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Reversibility of Electron Transfer in Tryptophan−Tyrosine Peptide in Acidic Aqueous Solution Studied by Time-Resolved CIDNP

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Time-resolved chemically induced dynamic nuclear polarization (CIDNP) has been used to study electron transfer reactions in tryptophan−tyrosine peptide under strongly acidic conditions. It is demonstrated that a decrease in pH from 2.4 to 1.6 reduces the overall efficiency of intramolecular electron transfer from the tyrosine residue to the oxidized tryptophan residue. A detailed analysis of the CIDNP kinetics revealed that the rate constant of this reaction k_f stays unchanged upon pH variation, whereas the rate constant of electron transfer in the opposite direction k_r increases with decreasing pH. The values of the rate constants extracted from model simulations are as follows: $k_f = (5.5 \pm 0.5) \times 10^5\ s^{-1}$; $k_r = (5.5 \pm 1.0) \times 10^4\ s^{-1}$ at pH 2.4, $(1.2 \pm 0.2) \times 10^5\ s^{-1}$ at pH 2.0, and $(3.2 \pm 0.4) \times 10^5\ s^{-1}$ at pH 1.6. The pH dependence of $\log K = \log(k_f/k_r)$ is linear and allows for the determination of the difference between the one-electron reduction potentials of the tryptophanyl and tyrosyl radicals in the peptide. The efficiency of IET in acidic aqueous solution containing 10 M urea-d_4 was estimated.

Introduction

The application of chemically induced dynamic nuclear polarization (CIDNP) to the study of proteins allows for the determination of their structural and dynamic properties.[1−12] CIDNP manifests itself in the anomalous intensities (enhanced absorption or emission) of signals in the NMR spectra of products of the termination of radicals formed via reaction of reversible electron (hydrogen) transfer between a triplet excited dye and CIDNP-active amino acid residues in a protein.[13−15] Three of the 20 common amino acids give rise to a significant CIDNP effect. They are tryptophan, tyrosine, and histidine.[1,3,13] Nuclear polarization is formed for the protons of these amino acids and for those of the dye during the nuclear spin dependent singlet−triplet transitions in the intermediate short-lived radical pairs.[15]

The majority of photo-CIDNP studies on proteins has been performed using the so-called continuous-wave detection mode (cw-CIDNP) that measures the stationary polarization when radical reactions are already completed.[1−12,16] The intensity of CIDNP signals in these experiments depends strongly on the rates and mechanisms of the reactions of radicals that escaped geminate termination and on the paramagnetic relaxation time of polarized nuclei in the radicals.[17−23] The contributions to polarization from the geminate and homogeneous stages of the reactions can be separated by application of time-resolved CIDNP with pulsed laser initiation of the photochemical reaction in the probe of the NMR spectrometer followed by a synchronized delayed pulsed NMR detection of the polarized reaction products.[17−23]

We have already shown that in native and denatured proteins CIDNP is time dependent on a microsecond scale.[21,24] Among the processes that affect the kinetics of nuclear polarization in proteins is the reaction of intramolecular electron transfer (IET) which involves tryptophan and tyrosine residues.[21] This reaction has been extensively investigated in a number of peptides and proteins using pulse radiolysis techniques.[25−31] The peculiarities of CIDNP formation in the radical reaction accompanied by IET were studied on the tryptophan−tyrosine peptide,[22,23] in which the efficiency of IET is pH-dependent.[26] It was demonstrated that in neutral (pH 7.4) and basic (pH 11.4) aqueous solution the IET from the tyrosine residue to the tryptophan radical competes with nuclear paramagnetic relaxation (several units of $10^4\ s^{-1}$).[23] In acidic solution (pH 3.0, 3.8), IET reaction

$$\mathrm{TrpH^{+\bullet}{-}TyrOH} \xrightarrow{k_f} \mathrm{TrpH{-}TyrO^{\bullet} + H^{+}} \qquad (1)$$

is much faster and competes with the second-order radical termination (several units of $10^5\ s^{-1}$).[22,23]

In proteins, this reaction of electron transfer is known to be efficient, for example, for the temperature denatured protein hen egg white lysozyme at pH 3.8, whose CIDNP kinetics closely resembles that of tryptophan−tyrosine peptide in acidic solution.[21]

It was reported that, under strong acidic or basic conditions, the one-electron reduction potential of electron-deficient tyrosine becomes higher than that of tryptophan, and electron deficiency is expected to proceed in the direction opposite to that in reaction 1.[32−34] However, in the literature there is no experimental evidence of the reversibility of reaction 1 in acidic solution. In this paper, we present a time-resolved study of CIDNP formation in the photoreaction of the tryptophan−tyrosine peptide with the aza-aromatic dye 2,2′-dipyridyl (DP) under strongly acidic conditions (pH < 2.5). Dipyridyl is found to be more suitable for the studies of transformations of tryptophan and tyrosine radicals than the traditionally used dye flavin mononucleotide (FMN): the CIDNP enhancement factors in the pairs of radicals

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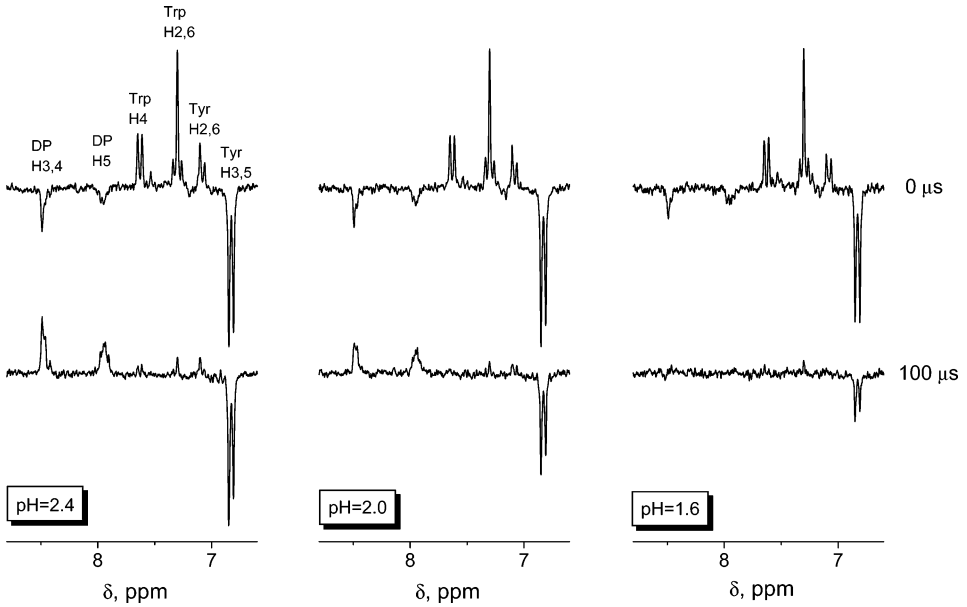
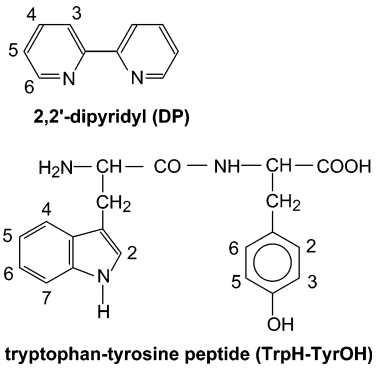


Figure 1. Aromatic region of ^{1}H CIDNP spectra obtained in photoreactions of 2,2′-dipyridyl with tryptophan−tyrosine peptide at pH 2.4 (left), pH 2.0 (middle), and pH 1.6 (right). Upper spectra were taken immediately after the laser pulse, lower spectra with a delay of 100 μs after the laser pulse.

CHART 1



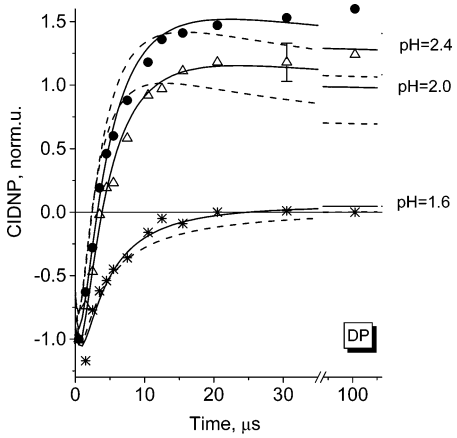


Figure 2. ^{1}H CIDNP kinetics for the protons H3,4 of 2,2′-dipyridyl obtained in photoreactions of 2,2′-dipyridyl with the peptide tryptophan−tyrosine at pH 2.4 (●), pH 2.0 (△), and pH 1.6 (∗). Lines show simulations using eqs 8−12 according to the procedure described in the text with the following parameters: solid lines, $-$ $k_f = 5.5 \times 10^5$ s^{-1}, $R_0k_1 = 2.2 \times 10^5$ s^{-1}, and $k_r = 5.5 \times 10^4$ s^{-1} (upper curve), $k_r = 1.2 \times 10^5$ s^{-1} (middle curve), and $k_r = 3.2 \times 10^5$ s^{-1} (lower curve); dashed lines, $k_r = 0$, and $k_f = 5.5 \times 10^5$ s^{-1}, $R_0k_1 = 3.6 \times 10^5$ s^{-1} (upper curve), $k_f = 5.0 \times 10^5$ s^{-1}, $R_0k_1 = 6.0 \times 10^5$ s^{-1} (middle curve), and $k_f = 6.0 \times 10^4$ s^{-1}, $R_0k_1 = 1.2 \times 10^6$ s^{-1} (lower curve). The parameters $k_1/k_2 = 1.25$, $\alpha = 0.6$, $T_1 = 100$ μs, and $\gamma = 2.8$ are common for all curves.

originating from dipyridyl/tryptophan and dipyridyl/tyrosine are comparable in magnitude[22] whereas for FMN polarization is much more efficiently formed in the pair with the tryptophanyl radical.[21] As a consequence, the CIDNP kinetics obtained with FMN is less sensitive to a change in concentration of the tyrosyl radical. The details of photochemical reactions of dipyridyl with tryptophan and tyrosine, as well as the peculiarities of CIDNP formation for these amino acids necessary for the present investigation have been studied earlier.[18,19] The knowledge about the direction and efficiency of IET between neighboring tryptophan and tyrosine residues is, in particular, important for the analysis of the CIDNP kinetic behavior of nonnative states of bovine and human α-lactalbumins at pH 2.

The structure of the compounds under study is shown in Chart 1.

Experimental Section

A detailed description of our TR-CIDNP setup was given previously.[24] The samples, sealed in a standard 5 mm NMR Pyrex ampule, were irradiated by a COMPEX Lambda Physik excimer laser (wavelength 308 nm, pulse energy up to 150 mJ) in the probe of a 200 MHz Bruker DPX-200 NMR spectrometer. Light was guided to the sample using an optical system containing a quartz lens, a prism, and a cylindrical light guide (5 mm diameter). TR-CIDNP experiments were carried out by applying the following pulse sequence: radio frequency (RF) saturation pulses−laser pulse−evolution time τ−RF detection pulse−free induction decay. Since the background signals in the spectrum originating from Boltzmann polarization are suppressed, only resonances from the polarized products formed during the variable delay τ appear in the CIDNP spectra. In all kinetic measurements, an RF pulse with duration of 1 μs was used for detection. The timing corresponds to the center of the RF pulse (i.e., 0.5 μs for $\tau = 0$) on all plots in Figures 2−4.

Each of the three sets of kinetic data (at pH 1.6, 2.0, 2.4) was recorded using 16 samples: each sample was used to acquire four scans for each of 13 τ values, so that every data point in Figures 2−4 represents 64 signal accumulations. The sample depletion after the 52 light flashes was never more than

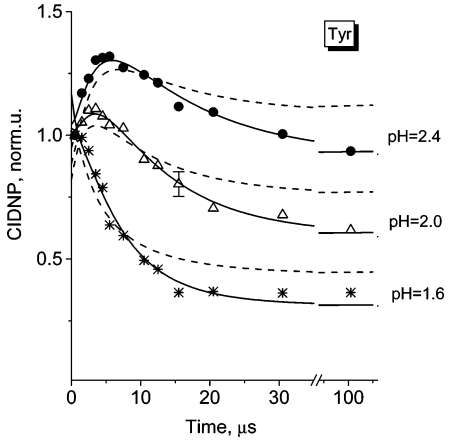


Figure 3. ^{1}H CIDNP kinetics for the protons H3,5 of the tyrosine residue obtained in photoreactions of 2,2′-dipyridyl with the peptide tryptophan−tyrosine at pH 2.4 (●), pH 2.0 (△), and pH 1.6 (*). Lines show simulations using eqs 8−10 and 16−18 according to the procedure described in the text with the following parameters: solid lines, $k_f = 5.5 \times 10^5$ s^{-1}, $R_0k_1 = 2.2 \times 10^5$ s^{-1}, $k_{ex} = 9 \times 10^5$ s^{-1}, and $k_r = 5.5 \times 10^4$ s^{-1} (upper curve), $k_r = 1.2 \times 10^5$ s^{-1} (middle curve), and $k_r = 3.2 \times 10^5$ s^{-1} (lower curve); dashed lines, $k_r = 0$, and $k_f = 5.5 \times 10^5$ s^{-1}, $R_0k_1 = 3.6 \times 10^5$ s^{-1} (upper curve), $k_f = 5.0 \times 10^5$ s^{-1}, $R_0k_1 = 6.0 \times 10^5$ s^{-1} (middle curve), and $k_f = 6.0 \times 10^4$ s^{-1}, $R_0k_1 = 1.2 \times 10^6$ s^{-1} (lower curve). The parameters $k_1/k_2 = 1.25$, $\alpha = 0.6$, $T_1 = 33$ μs, and $\gamma = 1.4$ are common for all curves.

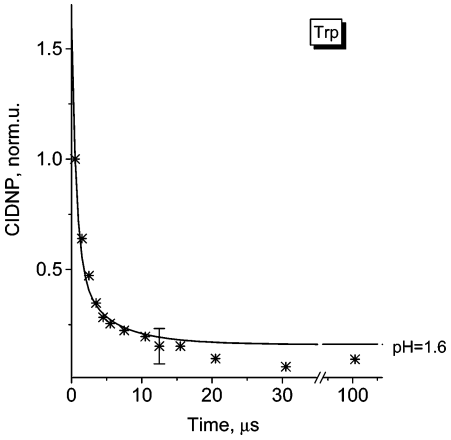


Figure 4. ^{1}H CIDNP kinetics for the protons H2,6 of the tryptophan residue obtained in photoreactions of 2,2′-dipyridyl with the peptide tryptophan−tyrosine at pH 1.6 (*). Line is simulation using eqs 8−10 and 19−21 according to the procedure described in the text. Parameters of simulations are $k_f = 5.5 \times 10^5$ s^{-1}, $R_0k_1 = 2.2 \times 10^5$ s^{-1}, $k_1/k_2 = 1.25$, $k_{ex} = 9 \times 10^5$ s^{-1}, $\alpha = 0.6$, $T_1 = 26$ μs, $\gamma = 2.8$, and $k_r = 3.2 \times 10^5$ s^{-1}.

30%. To avoid any distortion of the kinetics from this source, ascending and descending orders of the time delays were used alternately.

L-Tryptophan-L-tyrosine, 2,2′-dipyridyl (DP), urea-d_4, and D_2O were used as received from Sigma-Aldrich.

Concentrations were 5 mM for the peptide and 0.5 mM for 2,2′-dipyridyl. The pH of the NMR samples was adjusted by addition of DCl. No correction was made for the deuterium isotope effect on the pH.

Results and Discussion

Reaction Mechanism. The mechanism of quenching of triplet excited 2,2′-dipyridyl by both tryptophan and tyrosine under acidic conditions is electron transfer.[18,19] The quenching rate constants measured for the *N*-acetyl derivatives of the two amino acids were pH-independent at 1.4 < pH < 4.0 with the values k_q(TrpH) = 4.7 × 10^9 M^{-1} s^{-1} and k_q(TyrOH) = 3.4 × 10^9 M^{-1} s^{-1}.[18,19] The rate constant of dipyridyl quenching by the tryptophan−tyrosine peptide was found to be 2.5 × 10^9 M^{-1} s^{-1} at pH 3.8.[22] From the optical measurements, the partial contribution of the individual residues to the total quenching could not be determined. However, a close similarity of the distribution of line intensities in the CIDNP spectra detected immediately after the laser pulse for the peptide and the equimolar mixture of *N*-acetyl tryptophan and *N*-acetyl tyrosine indicated that the ratio of the quenching rate constants for the covalently linked residues is the same as that for the nonlinked amino acids.[22]

The quenching reactions of dipyridyl protonated at the acidic pH are written as

$$DPH^+ \xrightarrow{h\nu,\mathrm{ISC}} {}^TDPH^+ \quad (2)$$

$${}^TDPH^+ + TrpH{-}TyrOH \xrightarrow{k_q(TyrOH)} DPH^\bullet + TrpH^{+\bullet}{-}TyrOH \quad (3)$$

$${}^TDPH^+ + TrpH{-}TyrOH \xrightarrow[-H^+]{k_q(TrpH)} DPH^\bullet + TrpH{-}TyrO^\bullet \quad (4)$$

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

The cation radical of tyrosine, $TyrOH^{+\bullet}$, resulting from quenching via electron transfer, undergoes very fast deprotonation ($pK_a < -1$),[35] whereas $DPH^\bullet$ transforms into $DPH_2^{+\bullet}$ with $pK_a = 8.5$.[20]

At the concentration of the peptide, C_0, used in this work (5 mM), the characteristic time of quenching is $k_q^{-1}C_0^{-1} = 80$ ns, which is much shorter than the time resolution of the set up (1 μs). Thus, under these conditions, the radical formation can be considered as instantaneous, and the CIDNP kinetics presented in this work are not complicated by the time evolution of the triplet quenching process.

The choice of the concentration of 5 mM was caused by the necessity to overcome the problem of sample depletion, which was too high at pH 1.6 when a lower peptide concentration was used. The question arises whether intermolecular radical reactions between tryptophan and tyrosine residues are important at a peptide concentration of 5 mM. In earlier studies on mixtures of *N*-acetyl tryptophan and *N*-acetyl tyrosine at concentrations of 3 mM each, such reactions were not observable.[23] Since the diffusion rate of the peptide is lower than that of the two *N*-acetyl amino acids, intermolecular electron transfer under the conditions used in the present study is not expected. This is also confirmed by the agreement of the results of the present study obtained without taking into cosideration the intermolecular electron transfer with the results of previous investigations[22,23] (vide infra).

CIDNP Spectra. Figure 1 shows the aromatic region of the ^{1}H CIDNP spectra of the tryptophan−tyrosine peptide with DP at pH 2.4 (left), 2.0 (middle), and 1.6 (right). The upper spectra were recorded immediately after the laser pulse ($\tau = 0$) and the lower ones at $\tau = 100$ μs. For some reasons the signal-to-noise ratio significantly decreased upon decreasing the pH, and as a result, the quantitative analysis of CIDNP measurements at pH < 1.6 was impossible.

The CIDNP signs for the protons of the amino acid residues are in accordance with Kaptein's rules:[15] enhanced absorption of the H2,6, H4 of tryptophan and H2,6 of tyrosine and emission of H3,5 of tyrosine. Nuclear polarization of the protons of dipyridyl is formed in two radical pairs with different partner

radicals, $TrpH^{+\bullet}$ and $TyrO^{\bullet}$. The *g*-factor values increase in the sequence $g(TrpH^{+\bullet}) < g(DPH_2^{+\bullet}) < g(TyrO^{\bullet})$.[36−38] According to Kaptein's rule, the sign of polarization is determined by the sign of the *g*-factor difference of the partner radicals: the dye radical and the radical of the amino acid.[15,39] Thus, polarization of opposite sign is formed for dipyridyl in the two types of geminate radical pairs: emission in the radical pair with tryptophanyl and enhanced absorption in the pair with tyrosyl. The emission detected for dipyridyl immediately after the laser pulse is determined by two factors: (1) the predominance of the radical pairs with tryptophanyl due to a more efficient quenching by electron transfer from the tryptophan residue and (2) the CIDNP enhancement factor being 1.1 times larger in the pair with tryptophanyl than in that with tyrosyl.[22]

The CIDNP spectra detected immediately after the laser pulse at different pH are very similar, whereas a pronounced difference is observed in the spectra obtained at 100 μs after the laser pulse. In the upper spectra, the phase of CIDNP of dipyridyl is always emissive. At $\tau = 100$ μs and pH = 2.4, positive polarization is observed for the protons of the dye. A decrease of pH leads to a decrease of this positive polarization, which is accompanied by a reduction of tyrosine CIDNP at $\tau = 100$ μs. A change in sign of dipyridyl polarization from negative to positive reflects an increase in the contribution of radical pairs with tyrosine formed later via IET which was studied in detail previously in the photoreaction with the same peptide under acidic conditions at pH 3.8.[22] The sign change of the CIDNP of the dye indicates a high efficiency of reaction 1 with a rate comparable to that of second-order radical termination.[22] Since IET leads to additional formation of the tyrosyl radical, the stationary CIDNP value of tyrosine in the peptide is higher compared to that for tyrosine not linked to tryptophan, i.e., for inefficient electron transfer. For tryptophan, at acidic pH a fast decay of CIDNP is observed irrespective of linkage to the peptide. This is determined by the reaction of degenerate electron exchange, which leads to the transfer of polarization from radicals to ground-state molecules; the sign of transferred polarization is opposite to the sign of geminate CIDNP:[18]

$$*TrpH^{+\bullet} + TrpH \xrightarrow{k_{ex}} *TrpH + TrpH^{+\bullet} \qquad (6)$$

In eq 6, the asterisk denotes nuclear polarization. Since the concentration of the initial compound is much higher than that of the radicals, we consider the degenerate electron exchange as an irreversible pseudo first-order reaction with its rate constant proportional to the concentration of molecules involved in the exchange: $k_{ex} = k_{ex}^{bi}C_0$, where k_{ex}^{bi} is the second-order rate constant of degenerate electron exchange. Below, k_{ex} always denotes the pseudo first-order rate constant.

CIDNP Kinetics. The CIDNP kinetics of the most intensively polarized protons of dipyridyl (H3,4), tyrosine (H3,5), and tryptophan (H2,6) are shown in Figures 2−4, respectively. H2 and H6 of tryptophan are not magnetically equivalent in the intermediate radical. However, in most cases, they are superimposed; therefore, they cannot be separated in the NMR spectrum. The signal intensity detected immediately after the laser pulse (first point in the kinetics) was scaled to unity for each kinetics. For H2,6 of tryptophan and H3,5 of tyrosine, the absolute value of line intensities is plotted; for dipyridyl the sign of polarization is also shown.

The CIDNP observations correspond qualitatively to a decrease in the efficiency of IET (1) with decreasing pH. To analyze quantitatively the IET process, we simulated the CIDNP kinetics following the approach suggested by Vollenweider and Fischer,[17] but with the modification that three types of radicals are present in the solution. Two of them are interconverted by IET.[21] The attempt to describe the CIDNP kinetics treating IET as irreversible and slowing down with decreasing pH failed completely as seen from the best-fit simulations that are shown in Figures 2 and 3 by dashed lines. The corresponding values of k_f are 5.5×10^5 s^{-1} (pH 2.4), 5.0×10^5 s^{-1} (pH 2.0), and 6.0×10^4 s^{-1} (pH 1.6). In addition, the second-order rates of radical termination used in the simulations (details are given below) had to be varied significantly to fit the kinetics at different pH. Since the quenching rate constants are not pH-dependent in the pH range from 1.6 to 2.4, it is unlikely that the initial radical concentrations are noticeably different. The products of the second-order termination rate constant and the initial radical concentration were 3.6×10^5 s^{-1} (pH 2.4), 6.0×10^5 s^{-1} (pH 2.0), and 1.2×10^6 s^{-1} (pH 1.6). When the experimentally observed value of the second-order termination rate constant, 1.2×10^9 M^{-1} s^{-1},[22] is used we obtain the initial radical concentrations 3.0×10^{-4} M (pH 2.4), 5.0×10^{-4} M (pH 2.0), and 1.0×10^{-3} M (pH 1.6). The second and the third values are unrealistic, since they are equal to (5.0×10^{-4} M) or even higher (1.0×10^{-3} M) than the concentration of the dye in our solutions (5.0×10^{-4} M).

Thus, it became clear that electron transfer in the opposite direction has to be taken into account:

$$TrpH^{+\bullet}-TyrOH \underset{k_r}{\overset{k_f}{\rightleftarrows}} TrpH-TyrO^{\bullet} + H^+ \qquad (7)$$

Under such assumption the concentrations of the radicals present in the solution, R_{DP} (dipyridyl radical), R_{Trp} (tryptophanyl radical), and R_{Tyr} (tyrosyl radical), are determined by the following equations:

$$\frac{dR_{DP}}{dt} = -k_1R_{DP}R_{Trp} - k_2R_{DP}R_{Tyr} \qquad (8)$$

$$\frac{dR_{Trp}}{dt} = -k_1R_{DP}R_{Trp} - k_fR_{Trp} + k_rR_{Tyr} \qquad (9)$$

$$\frac{dR_{Tyr}}{dt} = -k_2R_{DP}R_{Tyr} + k_fR_{Trp} - k_rR_{Tyr} \qquad (10)$$

Here, k_1 and k_2 are the second-order termination rate constants for the radicals $DPH_2^{+\bullet}/TrpH^{+\bullet}-TyrOH$ and $DPH_2^{+\bullet}/TrpH-TyrO^{\bullet}$, respectively. The termination rate constants measured for *N*-acetyl derivatives of the amino acids are $k_1 = 2.0 \times 10^9$ M^{-1} s^{-1} and $k_2 = 1.6 \times 10^9$ M^{-1} s^{-1}.[18,19] In the present work, we used $k_1/k_2 = 1.25$, taken from the above values. When neglecting this difference, we can use algebraic instead of differential equations; this, however, leads to a worse agreement between calculations and experimental data. Thus, in the present work we took into account all the details of the system under study as obtained in the previous investigations.

In eqs 8−10, we neglected the fraction of geminate recombination and considered the formation of radicals as instantaneous. The initial concentrations of radicals are R_0 for $DPH_2^{+\bullet}$, αR_0 for $TrpH^{+\bullet}-TyrOH$, and $(1 - \alpha)R_0$ for $TrpH-TyrO^{\bullet}$ with $\alpha = k_q(TrpH)/[k_q(TrpH) + k_q(TyrOH)] = 0.6$.

Polarization in the dipyridyl radical (P^R) and the ground-state dipyridyl (P) is written as follows:

$$\frac{dP^R}{dt} = -k_1 P^R R_{Trp} - k_2 P^R R_{Tyr} - k_1\beta_1 R_{DP} R_{Trp} - k_2\beta_2 R_{DP} R_{Tyr} - \frac{P^R}{T_1} \quad (11)$$

$$\frac{dP}{dt} = k_1 P^R R_{Trp} + k_2 P^R R_{Tyr} + k_1\beta_1 R_{DP} R_{Trp} + k_2\beta_2 R_{DP} R_{Tyr} \quad (12)$$

In eqs 11 and 12, two types of radical pairs are considered. The first and second terms describe polarization transfer from the radical to the ground-state molecule in the termination with the radicals $TrpH^{+\bullet}-TyrOH$ and $TrpH-TyrO^{\bullet}$, respectively. The third and forth terms refer to polarization formation in radical pairs in the bulk, i.e., the so-called F-pairs. The last term in eq 11 describes nuclear paramagnetic relaxation with the time T_1. The polarization, created in F-pairs, is related to the parameter β proportional to the value of geminate polarization P^G: $\beta = \gamma P^G/R_0$. The quantity γ is the ratio of CIDNP created in geminate and in F-pairs, and it is believed to be equal to 3 in the case of a triplet precursor.[17] For modeling the formation of CIDNP for dipyridyl in the two types of radical pairs, we introduced the values β_1 and β_2 in eqs 11 and 12: $\beta_1 = \gamma P^{G1}/(\alpha R_0)$, $\beta_2 = \gamma P^{G2}/((1-\alpha)R_0)$. In accordance with the spin-sorting nature of the mechanism of CIDNP formation at high magnetic field, the initial conditions are $P(0) = -P^R(0) = P^{G1} + P^{G2}$. Since CIDNP is measured in arbitrary units, in our simulations $P(0)$ appears as a scaling factor. The ratio of nuclear polarization per radical pair, formed for dipyridyl in the radical pairs with tryptophanyl with respect to those with tyrosyl, is 1.1, as was found for the dipeptide and the equimolar mixture of the amino acids,[22] i.e.: $P^{G1}/\alpha \div P^{G2}/(1-\alpha) = 1.1$. We used this equation to reduce the number of fitting parameters and to express the scaling factor P^{G2} as a multiple of the scaling factor P^{G1}.

The rate constants k_f and k_r do not explicitely show up in eqs 11 and 12; however, the reactions of IET have influence on the CIDNP kinetics of dipyridyl via the radical concentrations R_{Trp} and R_{Tyr}.

The description of the CIDNP kinetics for the protons of the amino acid residues is more complex. The degenerate electron exchange leads to polarization transfer from tryptophan-centered peptide radicals to ground-state peptides (polarization is denoted by the asterisk):

$$*TrpH^{+\bullet}-TyrOH + TrpH-TyrOH \xrightarrow{k_{ex}} *TrpH-TyrOH + TrpH^{+\bullet}-TyrOH \quad (13)$$

In addition, the degenerate electron exchange has influence on the polarization of the tyrosine moiety: tryptophan-centered peptide radicals with polarization of their tyrosine protons are converted into ground-state peptides with polarized tyrosine residues

$$TrpH^{+\bullet}-*TyrOH + TrpH-TyrOH \xrightarrow{k_{ex}} TrpH-*TyrOH + TrpH^{+\bullet}-TyrOH \quad (14)$$

The particles on the right- and on the left-hand sides of eq 14 contribute differently to the CIDNP kinetics with respect to electron transfer:

$$\begin{array}{ccc} TrpH^{+\bullet}-*TyrOH & \underset{k_f}{\overset{k_r}{\rightleftharpoons}} & TrpH-*TyrO^{\bullet} + H^+ \\ \downarrow k_{ex} & & \\ TrpH^{+\bullet}-TyrOH & \underset{k_f}{\overset{k_r}{\rightleftharpoons}} & TrpH-TyrO^{\bullet} + H^+ \end{array} \quad (15)$$

Thus, the reversibility of electron transfer together with the presence of degenerate electron exchange requires that polarization be considered in three rather than two types of particles connected by a corresponding relationship. Equations 16−18 describe the polarization of tyrosine protons in the tyrosine-centered radical $TrpH-TyrO^{\bullet}$ (P^R_{Tyr}), the tryptophan-centered radical $TrpH^{+\bullet}-TyrOH$ (P^r_{Tyr}), and the ground-state peptide $TrpH-TyrOH$ (P_{Tyr}):

$$\frac{dP^R_{Tyr}}{dt} = -k_2 P^R_{Tyr} R_{DP} - k_2\beta_{Tyr} R_{DP} R_{Tyr} + k_f P^r_{Tyr} - k_r P^R_{Tyr} - \frac{P^R_{Tyr}}{T_1} \quad (16)$$

$$\frac{dP^r_{Tyr}}{dt} = -k_1 P^r_{Tyr} R_{DP} - k_{ex} P^r_{Tyr} - k_f P^r_{Tyr} + k_r P^R_{Tyr} \quad (17)$$

$$\frac{dP_{Tyr}}{dt} = k_2 P^R_{Tyr} R_{DP} + k_2\beta R_{DP} R_{Tyr} + k_1 P^r_{Tyr} R_{DP} + k_{ex} P^r_{Tyr} \quad (18)$$

Equations 16−18 contain not only the terms of second-order radical termination and paramagnetic relaxation but also the terms relating to the polarization transfer in the reactions of IET and degenerate electron exchange. The chemical shift of tyrosine protons in the peptide with electron deficient tryptophan can differ from that in the diamagnetic peptide due to a small Knight shift. However, the FT-NMR signal acquisition starts 250 μs after the excitation pulse, when all radicals have decayed, hence the polarizations P^r and P are detected together. Thus, the experimental points in Figure 3 correspond to $P^r_{Tyr} + P_{Tyr}$. The initial conditions are as follows: $P_{Tyr}(0) = -P^R_{Tyr}(0) = P^G$, $P^r_{Tyr}(0) = 0$.

The polarization of tryptophan protons in particles with the radical center at the tryptophan moiety (P^R_{Trp}), with the radical center at the tyrosine moiety (P^r_{Trp}), and in the ground-state peptide (P_{Trp}) can be written as

$$\frac{dP^R_{Trp}}{dt} = -k_1 P^R_{Trp} R_{DP} - k_1\beta R_{DP} R_{Tyr} - k_{ex} P^R_{Trp} - k_f P^R_{Trp} + k_r P^r_{Trp} - \frac{P^R_{Trp}}{T_1} \quad (19)$$

$$\frac{dP^r_{Trp}}{dt} = -k_2 P^r_{Trp} R_{DP} + k_f P^R_{Trp} - k_r P^r_{Trp} \quad (20)$$

$$\frac{dP_{Trp}}{dt} = k_1 P^R_{Trp} R_{DP} + k_1\beta R_{DP} R_{Trp} + k_2 P^r_{Trp} R_{DP} + k_{ex} P^R_{Trp} \quad (21)$$

Experimental points in Figure 4 correspond to the value $P^r_{Trp} + P_{Trp}$ as described by eqs 19−21.

The parameters used in the simulations of the CIDNP kinetics that are known from previous measurements are as follows. For the parameter γ we used the value 2.8, except for tyrosine, for which $\gamma = 1.4$ was found.[19] The paramagnetic relaxation times were 26 μs for H2,6 of tryptophan and 33 μs for H3,5 of tyrosine.[21] A relaxation time of 100 μs was taken for dipyridyl

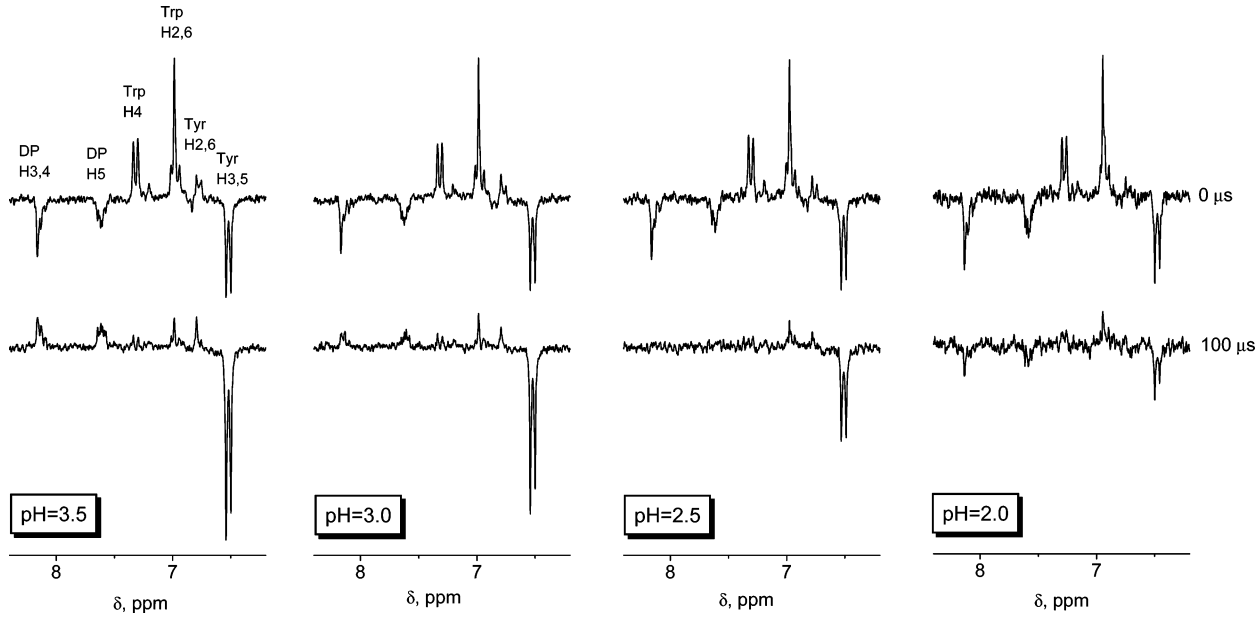


Figure 5. Aromatic region of ^{1}H CIDNP spectra, obtained in photoreactions of 2,2′-dipyridyl with the peptide tryptophan−tyrosine at pH 3.5, 3.0, 2.5, 2.0 (left to right) in the presence of 10 M urea-d_4. Upper spectra were taken immediately after the laser pulse; lower spectra were taken 100 μs after the laser pulse.

TABLE 1: Rate Constants k_f and k_r of Intramolecular Electron Transfer in the Tryptophan−Tyrosine Peptide and Equilibrium Constant $K = k_f/k_r$[a]

pH	k_f, s^{-1}	k_r, s^{-1}	$K = k_f/k_r$	10 M urea-d_4
2.4	$(5.5 \pm 0.5) \times 10^5$	$(5.5 \pm 1.0) \times 10^4$	10.0 ± 2.5	−
2.0		$(1.2 \pm 0.2) \times 10^5$	4.6 ± 1.2	
1.6		$(3.2 \pm 0.4) \times 10^5$	1.7 ± 0.4	
3.5	$(3.0 \pm 0.5) \times 10^5$	$(6.0 \pm 0.5) \times 10^4$	5.0 ± 1.3	+
3.0		$(8.0 \pm 0.5) \times 10^4$	3.8 ± 0.8	
2.5		$(1.5 \pm 0.2) \times 10^5$	2.0 ± 0.6	
2.0		$(2.2 \pm 0.1) \times 10^5$	1.4 ± 0.3	

[a] For definitions of k_f and k_r see eq 7.

instead of the previously found value of 44 μs.[18] The latter value corresponds to the $DPH^•$ radical in basic solution, whereas for the radical DPH_2^+ ($pK_a = 8.5$) which predominates in acidic solution, more detailed studies provided a higher value (100 μs).[40] As mentioned above, we fixed the parameter α at 0.6, and $k_1/k_2 = 1.25$.

Fitting parameters were R_0k_1, k_{ex}, and—varying with the value of pH—k_f and k_r. The best-fit simulations are shown in Figures 2−4 by solid lines. The best-fit parameters are as follows: $R_0k_1 = 2.2 \times 10^5$ s^{-1}, $k_{ex} = 9 \times 10^5$ s^{-1}, and the values of k_f and k_r that are listed in Table 1.

The freedom in the parameter variation was significantly reduced by the necessity to simultaneously fit the kinetics for three participating particles. For example, the CIDNP kinetics of dipyridyl was found to be very sensitive to k_f, and allowed this value to vary only in the narrow range between 5×10^5 s^{-1} and 6×10^5 s^{-1}. Thus, a value of 5.5×10^5 s^{-1} was chosen, which is in good agreement with the previously reported values 5×10^5 s^{-1} at pH = 3.8[22] and 6×10^5 s^{-1} at pH = 3.0.[23] Once the k_f was fixed, the kinetics of tyrosine was found to be very sensitive to k_r. Therefore, the freedom in its variation was also limited. The kinetics of tryptophan was less sensitive to the parameters, since a very fast decay of polarization determined by both, k_f and k_{ex} was observed. The kinetics obtained for tryptophan at different pH was very similar, differing slightly in the decay rate. In Figure 4, the slowest kinetics observed at pH 1.6 is shown together with a satisfactory fit with the above-mentioned parameters.

In addition, we made first studies of the peculiarities of IET in acidic solution ($2 < pH < 3.5$) containing 10 M urea-d_4. These conditions were chosen because in the presence of urea nonnative states of α-lactalbumins were obtained in aqueous solution at pH 2.[41] The tryptophan−tyrosine peptide represents a model of the highly efficient IET, providing the estimation of the upper limit of IET rate constant in denatured proteins.

The rate constants k_f and k_r were estimated from the analysis of CIDNP intensities of tyrosine and dipyridyl in the spectra obtained without delay after the laser pulse and with a delay of 100 μs when radical termination is over. The CIDNP spectra taken at 0 and 100 μs after the laser pulse for the peptide in the acidic solution in the presence of 10 M urea-d_4 are shown in Figure 5. The pH values were stepwise decreased from 3.5 to 2.0. It is seen that the spectra recorded immediately after the laser pulse are all very similar, as in the case when no urea was added to the solution. In the spectra taken at 100 μs after the laser pulse, the contribution of emissive polarization to the dipyridyl signal increases with decreasing pH: the absorptive polarization of DP at pH 3.5 is higher than that at pH = 3.0, while no polarization of DP is detected at pH 2.5, and the CIDNP is emissive at pH 2.0. As far as tyrosine is concerned, the value of its emissive polarization in the spectra taken at 100 μs becomes lower with increasing acidity. The changes in the polarization pattern for both dipyridyl and tyrosine reflect the shift of equilibrium between the two IET reactions toward the tryptophanyl radical. The dependencies of the polarization ratio $P(100\ \mu s)/P(0)$ for H3,4 of dipyridyl and for H3,5 of tyrosine on the pH are shown in Figure 6.

To extract the values of k_f and k_r under these conditions, we performed simulations of the polarization ratio $P(100\ \mu s)/P(0)$ for dipyridyl and tyrosine using the set of differential eqs 8−18. The viscosity of 10 M urea aqueous solution is twice as high as that of water,[42] and the parameters depending on viscosity were calculated using this relationship: $R_0k_1 = 1.1 \times 10^5$ s^{-1}, $k_{ex} = 4.5 \times 10^5$ s^{-1}, $T_1 = 17$ μs (H3,5 of the tyrosyl radical), and $T_1 = 50$ μs (H3,4 of the dipyridyl radical). The ratio of the quenching rate constants that determines the parameter α in urea differs from that in water. From the comparison of the relative intensities of tryptophan and tyrosine signals in the CIDNP

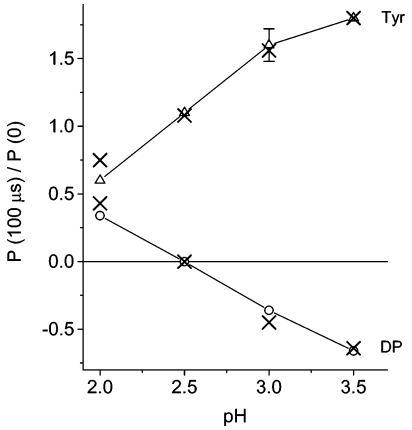


Figure 6. pH dependence of the ratio $P(100\ \mu s)/P(0)$ of CIDNP signals obtained at 100 μs and without delay after the laser pulse for the protons H3,4 of 2,2′-dipyridyl (○) and H3,5 of the tyrosine residue in the peptide (△). Crosses correspond to the values obtained from model simulations with the parameters $k_f = 3.0 \times 10^5\ s^{-1}$, $R_0k_1 = 1.1 \times 10^5\ s^{-1}$, $k_1/k_2 = 1.25$, $k_{ex} = 4.5 \times 10^5\ s^{-1}$, $\alpha = 0.7$, $T_1 = 17\ \mu s$ (tyrosine), $T_1 = 50\ \mu s$ (dipyridyl), $\gamma = 1.4$ (tyrosine), and $\gamma = 2.8$ (dipyridyl) common for all pH values and $k_r = 6.0 \times 10^4\ s^{-1}$ (pH 3.5), $k_r = 8.0 \times 10^4\ s^{-1}$ (pH 3.0), $k_r = 1.5 \times 10^5\ s^{-1}$ (pH 2.5), and $k_r = 2.2 \times 10^5\ s^{-1}$ (pH 2.0).

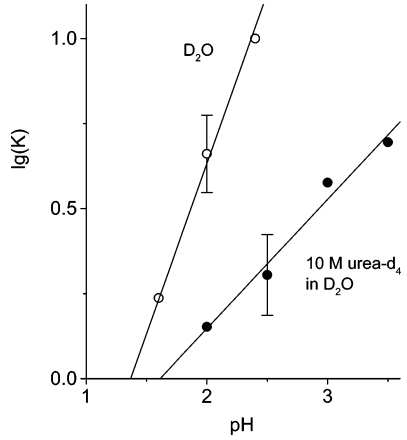


Figure 7. pH dependence of the equilibrium constant K of reaction 7: (○) in D_2O; (●) in D_2O containing 10 M-urea-d_4. The values of K are given in Table 1.

spectra obtained with and without urea, we estimated $\alpha = 0.7$. The crosses in Figure 6 denote the $P(100\ \mu s)/P(0)$ values calculated using the abovementioned parameter set and the values of k_f and k_r given in Table 1. It is seen the rates of IET from tyrosine to tryptophanyl radical (1) is slower than that in water, and the equilibrium is shifted toward the tryptophanyl radical.

Reduction Potentials of the Tryptophan and Tyrosine Radicals and pH Dependence of the Equilibrium Constant. The pH dependence of the reduction potential of one-electron oxidized tryptophan is described by the formula[43]

$$E^{Trp} = E_0^{Trp} + 0.059 \log \frac{[H^+]}{K_a^{Trp} + [H^+]} \qquad (22)$$

where E_0^{Trp} is the reduction potential at pH 0, and K_a^{Trp} is the ionization constant for the tryptophan cation radical ($pK_a = 4.3$ for free tryptophan,[44] and $4.6 < pK_a < 5.4$ for tryptophan in the peptides[43,45]). For the tyrosyl radical[34]

$$E^{Tyr} = E_0^{Tyr} + 0.059 \log(K_a^{Tyr} + [H^+]) \qquad (23)$$

where E_0^{Tyr} is the reduction potential at pH 0, and $pK_a = 10.1$ for the phenolic proton in free tyrosine and tyrosine in peptides.[43] In the pH range studied in the present work, the one-electron reduction potential of tryptophan remains constant, whereas that of tyrosine decreases by 0.059 V/pH. The values of k_f and k_r, extracted from our CIDNP simulations, qualitatively agree with the electrochemical properties of the system under study: k_f is pH-independent, and k_r decreases with increasing pH. The dependence of $\log K = \log(k_f/k_r)$ is shown in Figure 7 by open circles. The good linearity of the dependence between pH and $\log K$ (with slope equal to unity) proves that the obtained values of k_f and k_r are self-consistent and rather reliable (the correlation coefficient is 0.998).

The reduction potentials of the one-electron oxidized amino acids obtained by Harriman[33] using cyclic voltammetry are $E^{Trp} = 1.15$ V at pH 2 and $E^{Tyr} = 0.72$ V at pH 13. Taking into account the pH-dependencies given by eqs 22 and 23, we obtain that the equilibrium condition $K = 1$ for free tryptophan and tyrosine in the acidic region is met at pH 2.8, and the difference of reduction potentials at pH 0, $\Delta E_0 = E_0^{Trp} - E_0^{Tyr}$, is equal to -0.165 V. Linear extrapolation of our experimental data gives pH 1.4 for $K = 1$. In terms of reduction potentials, it means that the curves describing E^{Trp} and E^{Tyr} for the amino acids linked in the peptide are moved apart by an additional 0.059-(2.8 − 1.4) = 0.083 V in comparison with those for free amino acids, and $\Delta E_0 = -0.082$ V.

Data on ΔE for the tryptophan−tyrosine peptide at acidic pH are absent in the literature. However, knowing the pH-dependencies of the reduction potentials 22 and 23 and the ionization constants of the tryptophan cation radical and of tyrosine, we can calculate ΔE at pH 7 so as to be able to compare it with the estimate of 190 mV reported by Faraggi et al.[29] for the tryptophan−tyrosine peptide. The expression for the reduction potential difference

$$\Delta E = \Delta E_0 + 0.059 \log \frac{[H^+]}{(K_a^{Trp} + [H^+])(K_a^{Tyr} + [H^+])} \qquad (24)$$

at pH = 7 can be simplified to $\Delta E(pH = 7) = \Delta E_7 = \Delta E_0 + 0.059 pK_a^{Trp}$.

Using $\Delta E_0 = -0.082$ V and $pK_a^{Trp} = 4.6$, for ΔE_7 we will obtain the same 0.19 V as in ref 29.

For tryptophan and tyrosine inserted into peptides containing glycine, glutamate, lysine, proline, and alanine, DeFelippis and co-workers[43] found that the deviations of E^{Trp} and E^{Tyr} from the values obtained for free tyrosine and tryptophan did not exceed 0.02 V. The data of Faraggi et al.[29] and our results indicate that either tryptophan lowers the reduction potential of the tyrosyl radical or tyrosine raises the reduction potential of the tryptophanyl radical (or both) to a degree higher than that in the case when tryptophan and tyrosine are inserted into peptides with nonaromatic residues.

As calculated from known ΔE_0, pK_a^{Trp}, and pK_a^{Tyr}, the equilibrium condition $K = 1$ for the peptide in basic solution is achieved at pH 13.3, which is noticeably higher than pH 11.6 for the equilibrium of free amino acids.

For the aqueous solution containing 10 M urea, the dependence of $\log(K)$ on pH is linear with the slope 0.4 (solid circles in Figure 7). The deviation of the slope from unity may be caused either by specific interactions between peptide and urea, or by the difference between the real and the measured pH in aqueous solution containing 10 M urea.

Conclusion

This work was undertaken with the aim to perform a quantitative analysis of photoinduced bidirectional intramolecular electron transfer between tryptophan and tyrosine moieties under strongly acidic conditions using the high potential of time-resolved CIDNP in determination of radical reaction mechanisms and absolute rate constants. On the basis of results of our previous studies, a simple and well-characterized reaction system was chosen which is known to give strong effects of nuclear polarization. The CIDNP kinetics obtained in photoreactions of 2,2′-dipyridyl with the tryptophan−tyrosine peptide proved to be very sensitive to the intramolecular electron transfer (IET) in the peptide. In reactions with the two residues, the sign of the dye polarization (emissive in the pair with tryptophanyl and absorptive in pair with tyrosyl) is a characteristic and sensitive indicator that allows one to elicit the direction and efficiency of IET. Time-resolved CIDNP detection, which combines the kinetic possibilities of laser flash photolysis with the high spectral resolution of NMR, is suitable for providing a deep insight into the elementary steps in complex chemical reactions. It makes it possible to extract the rate constants of IET in both directions, k_f and k_r, from the tyrosine to the tryptophan radical moiety and from the tryptophan to the tyrosine radical moiety, respectively. In acidic solutions with pH varying from 1.6 to 2.4, k_f remains constant, while k_r changes. The difference between the one-electron reduction potentials of the tryptophanyl and tyrosyl radicals in the peptide was obtained from the value of the equilibrium constant $K = k_f/k_r$, which showed a good linear dependence on pH with a slope equal to unity. With the perspective of future applications of CIDNP to studies of protein folding from nonnative states formed in the presence of denaturing agents in acidic conditions, additional experiments were carried out in acidic solutions containing 10 M urea-d_4 at pH from 2.0 to 3.5. These parameter values were chosen to model the conditions under which nonnative states of the small globular protein α-lactalbumin can be obtained: at pH = 2, α-lactalbumins are known as a model system for the "molten globule" state,[46,47] and the addition of urea leads to the further loss of its tertiary structure.[41] Since α-lactalbumins contain a pair of neighboring tyrosine/tryptophan residues, Tyr103/Trp104, IET can be effective and should be taken into account in the analysis of CIDNP effects. Finally, the present results confirm that in the study of protein structure and dynamics exploitation of the full potential of the CIDNP method requires application of the time-resolved variant of the technique.

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References and Notes

(1) Kaptein, R.; Dijkstra, K.; Nicolay, K. *Nature (London)* **1978**, *274*, 293−294.

(2) Kaptein, R. Structural information from photo-CIDNP in proteins. In *NMR Spectroscopy in Molecular Biology*; Pullman, B., Ed.; D. Reidel: Dordrecht, The Netherlands, 1978; pp 211−229.

(3) Hore, P. J.; Broadhurst, R. W. *Prog. NMR Spectrosc.* **1993**, *25*, 345−402.

(4) Hore, P. J.; Kaptein, R. *Biochemistry* **1983**, *22*, 1906−1911.

(5) Kaptein, R. *Biol. Magn. Reson.* **1982**, *4*, 145−149.

(6) Wirmer, J.; Kuehn, T.; Schwalbe, H. *Angew. Chem.* **2001**, *113*, 4378−4380.

(7) Hore, P. J.; Winder, S. L.; Roberts, C. H.; Dobson, C. M. *J. Am. Chem. Soc.* **1997**, *119*, 5049−5050.

(8) Dobson, C. M.; Hore, P. J. *Nat. Struct. Biol., NMR Suppl.* **1998**, *5*, 504−507.

(9) Lyon, C. E.; Lopez, J. J.; Cho, B.-M.; Hore, P. J. *Mol. Phys.* **2002**, *100*, 1261−1269.

(10) Scheffler, J. E.; Cohn, M. *Biochemistry* **1986**, *25*, 3788−3796.

(11) Muszkat, K. A.; Khait, I.; Weinstein, S. *Biochemistry* **1984**, *23*, 5−10.

(12) Berliner, L. J.; Kaptein, R. *Biochemistry* **1981**, *20*, 799−807.

(13) Stob, S.; Kaptein, R. *Photochem. Photobiol.* **1989**, *49*, 565−577.

(14) *Chemically Induced Magnetic Polarization*; Muus, L. T.; Atkins, P. W.; McLauchlan, K. A.; Pedersen, J. B., Eds.; D. Reidel: Dordrecht, The Netherlands, 1977.

(15) Salikhov, K. M.; Molin, Y. N.; Sagdeev, R. Z.; Buchachenko, A. L. *Spin Polarization and Magnetic Field Effects in Radical Reactions*, Molin, Yu. N., Ed.; Elsevier: Amsterdam, 1984.

(16) Muszkat, K. A.; Weinstein, S.; Khait, I.; Vered, M. *Biochemistry* **1982**, *21*, 3775−3779.

(17) Vollenweider, J.-K.; Fischer, H. *Chem. Phys.* **1988**, *124*, 333−345.

(18) Tsentalovich, Y. P.; Morozova, O. B.; Yurkovskaya, A. V.; Hore, P. J. *J. Phys. Chem. A* **1999**, *103*, 5362−5368.

(19) Tsentalovich, Y. P.; Morozova, O. B. *J. Photochem. Photobiol. A: Chem.* **2000**, *30*, 33−40.

(20) Tsentalovich, Y. P.; Morozova, O. B.; Yurkovskaya, A. V.; Hore, P. J.; Sagdeev, R. Z. *J. Phys. Chem. A* **2000**, *104*, 6912−6916.

(21) Morozova, O. B.; Yurkovskaya, A. V.; Tsentalovich, Y. P.; Forbes, M. D. E.; Hore, P. J.; Sagdeev, R. Z. *Mol. Phys.* **2002**, *100*, 1187−1195.

(22) Morozova, O. B.; Yurkovskaya, A. V.; Tsentalovich, Y. P.; Forbes, M. D. E.; Sagdeev, R. Z. *J. Phys. Chem. B* **2002**, *106*, 1455−1460.

(23) Morozova, O. B.; Yurkovskaya, A. V.; Vieth, H.-M.; Sagdeev, R. Z. *J. Phys. Chem. B* **2003**, *107*, 1088−1096.

(24) Morozova, O. B.; Yurkovskaya, A. V.; Sagdeev, R. Z.; Mok, H. K.; Hore, P. J. *J. Phys. Chem. B* **2004**, *108*, 15355−15363.

(25) Prütz, W. A.; Butler, J.; Land, E. J.; Swallow, A. J. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 408−414.

(26) Prütz, W. A.; Land, E. J.; Sloper, R. W. *J. Chem. Soc., Faraday Trans. 1* **1981**, *77*, 281−292.

(27) Prütz, W. A.; Siebert, F.; Butler, J.; Land, E. J.; Menez, A.; Montenay-Garestier, T. *Biochim. Biophys. Acta* **1982**, *705*, 139−149.

(28) Butler, J.; Land, E. J.; Prütz, W. A.; Swallow, A. J. *Biochim. Biophys. Acta* **1982**, *705*, 150−162.

(29) Faraggi, M.; DeFelippis, M. R.; Klapper, M. H. *J. Am. Chem. Soc.* **1989**, *111*, 5141−5145.

(30) Bobrowski, K.; Holcman, J.; Poznanski, J.; Wierzchowski, K. L. *Biophys. Chem.* **1997**, *63*, 153−166.

(31) Bobrowski, K.; Poznanski, J.; Holcman, J.; Wierzchowski, K. L. *J. Phys. Chem. B* **1999**, *103*, 10316−10324.

(32) Butler, J.; Land, E. J.; Prütz, W. A.; Swallow, A. J. *J. Chem. Soc., Chem. Commun.* **1986**, 348−349.

(33) Harriman, A. *J. Phys. Chem.* **1987**, *91*, 6102−6104.

(34) Jovanovic, S. V.; Harriman, A.; Simic, M. *J. Phys. Chem.* **1986**, *90*, 1935−1939.

(35) Bent, D. V.; Hayon, E. *J. Am. Chem. Soc.* **1975**, *97*, 2612−2619.

(36) Grosse, S. Ph.D. Thesis, Free University of Berlin, 2000.

(37) Landolt-Boernstein *Magnetic Properties of Free Radicals*; Springer-Verlag: Berlin, Heidelberg, Germany, and New York, 1977.

(38) Koenig, E.; Fischer, H. *Z. Naturforsch.* **1962**, *17a*, 1063.

(39) Kaptein, R. *J. Chem. Soc., Chem. Commun.* **1971**, 732.

(40) Kiryutin, A. S. Diploma Thesis (in Russian), Novosibirsk State University, 2004.

(41) Wijesinha-Bettoni, R.; Dobson, C. M.; Redfield, C. *J. Mol. Biol.* **2001**, *312*, 261−273.

(42) *Handbook of Chemistry and Physics*; 81st ed.; Lide, D. R., Ed.; CRC Press: Boca Raton, FL, 2000.

(43) DeFelippis, M. R.; Murthy, C. P.; Proiman, F.; Weinraub, D.; Faraggi, M.; Klapper, M. H. *J. Phys. Chem.* **1991**, *95*, 3416.

(44) Posener, M. L.; Adams, G. E.; Wardman, P.; Cundall, R. B. *J. Chem. Soc., Faraday Trans. 1* **1976**, 2231−2239.

(45) Jovanovic, S. V.; Simic, M. G. *Free Radical Biol. Med.* **1985**, *1*, 125.

(46) Kuwajima, K. *FASEB J.* **1996**, *10*, 102−109.

(47) Kühn, T.; Schwalbe, H. *J. Am. Chem. Soc.* **2000**, *122*, 6169−6174.