ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/231630748

Time-Resolved CIDNP Study of Intramolecular Charge Transfer in the Dipeptide Tryptophan-Tyrosine

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · JANUARY 2002

Impact Factor: 3.3 · DOI: 10.1021/jp011858t

CITATIONS
22

READS
23

5 AUTHORS, INCLUDING:

Alexandra V Yurkovskaya
International Tomographic Center
122 PUBLICATIONS **1,379** CITATIONS
SEE PROFILE

Yuri Tsentalovich
International Tomographic Center
88 PUBLICATIONS **1,232** CITATIONS
SEE PROFILE

Malcolm D E Forbes
University of North Carolina at Chapel Hill
94 PUBLICATIONS **2,201** CITATIONS
SEE PROFILE

Available from: Alexandra V Yurkovskaya
Retrieved on: 23 January 2016

Time-Resolved CIDNP Study of Intramolecular Charge Transfer in the Dipeptide Tryptophan-Tyrosine

Olga B. Morozova, Alexandra V. Yurkovskaya,* Yuri P. Tsentalovich, Malcolm D. E. Forbes,† and Renad Z. Sagdeev

International Tomography Center, 630090, Institutskaya 3a, Novosibirsk, Russia, and Venable and Kenan Laboratories, Department of Chemistry, CB 3290, University of North Carolina, Chapel Hill, North Carolina 27599

Received: May 15, 2001; In Final Form: October 15, 2001

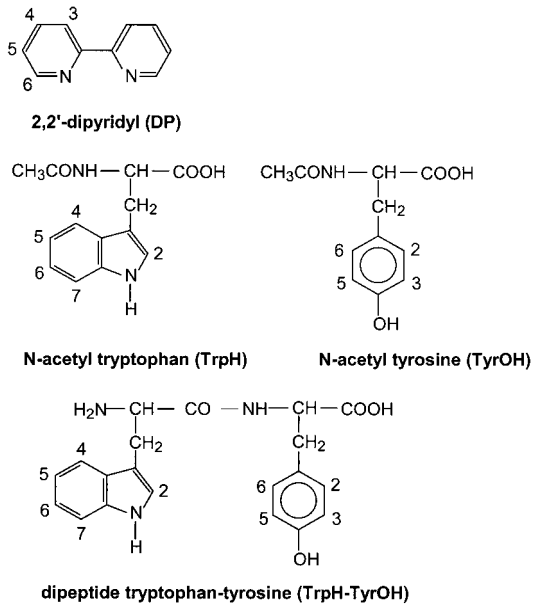
Time-resolved chemically induced dynamic nuclear polarization (CIDNP) and laser flash photolysis (LFP) techniques have been used to study the kinetics and mechanism of the photochemical reaction between 2,2′-dipyridyl (DP) and the dipeptide L-tryptophan-L-tyrosine (TrpH-TyrOH) in acidic aqueous solution (pH = 3.8). Analysis of the geminate CIDNP pattern reveals that the quenching of the protonated triplet dipyridyl $^{T}DPH^{+}$ results in the formation of both tryptophan and tyrosine radicals from the dipeptide with comparative efficiency. The total quenching rate constant of triplet dipyridyl by TrpH-TyrOH was found to be $(2.5 \pm 0.3) \times 10^{9}$ M^{-1} s^{-1}. The radical transformation $TrpH^{+\bullet} \rightarrow TyrO^{\bullet}$ via intramolecular electron transfer (IET) leads to an increasing tyrosyl radical concentration, growth of the tyrosine CIDNP signals, fast decay of the CIDNP signal of TrpH, and inversion of the CIDNP sign from emission to enhanced absorption for DP. The nuclear spin−lattice relaxation times of the radicals formed in the reactions and the rate constant for IET ($5 \times 10^{5} s^{-1}$) were determined by quantitative analysis of the CIDNP kinetics at different dipeptide concentrations in D_2O. A significant isotope effect ($k_H/k_D = 1.5$) was found for the IET rate constant by LFP measurements. In the presence of efficient IET, degenerate electron exchange between the $TrpH^{\bullet+}$ radical of the dipeptide and the diamagnetic molecule makes a negligible contribution to the decay of tryptophan CIDNP signal.

Introduction

In recent years, chemically induced dynamic nuclear polarization (CIDNP) has proven useful in the investigation of spatial structure of proteins in solution.[1−3] The method is based on the irradiation of an aqueous protein solution in the presence of a water soluble dye, followed by the NMR detection of CIDNP signals[4,5] (enhanced absorption or emission) arising due to reactions between the excited dye and accessible reactive groups in the biomacromolecule (amino acid side-chains). Only three amino acid residues (tryptophan, tyrosine, and histidine) are known to give rise to significant spin polarization. Since the dye can react only with residues exposed at the protein surface, a quantitative analysis of the CIDNP signals allows investigation of the solution structure of biological macromolecules.

The CIDNP intensities depend not only on the accessibility of the amino acid residues, but also on the mechanism of the photochemical reaction, the rate constants and mechanisms of the radical reactions in the bulk, and the rates of nuclear paramagnetic relaxation. Knowledge of these mechanisms and rate constants for individual amino acids is very important for the interpretation of CIDNP data obtained with biomacromolecules. We have recently performed a detailed study of the kinetics and mechanism of photochemical reactions of the aza-aromatic dye 2,2-dipyridyl (DP) with *N*-acetyl derivatives of CIDNP active amino acids (Chart 1), combining time-resolved CIDNP (TR−CIDNP) and laser flash photolysis (LFP).[6−8] It has been shown that, depending on the pH of the solution, the quenching of a triplet dye by an amino acid can proceed via either electron or hydrogen atom transfer, the latter being 1−2 orders of magnitude slower than the former. Formation of CIDNP is strongly affected by a degenerate electron exchange between the radicals and the ground-state molecules; the efficiency of this process is also pH-dependent. In that study, we also determined the absolute rate constants for the chemical

CHART 1



* To whom correspondence should be addressed.
† Venable and Kenan Laboratories.

10.1021/jp011858t CCC: $22.00 © 2002 American Chemical Society
Published on Web 01/22/2002

reactions, the nuclear relaxation times of the radicals involved, and achieved a quantitative description of the observed kinetics of CIDNP formation and decay.

However, the data obtained from these experiments with individual amino acids are not sufficient for a complete description of CIDNP in biomacromolecules. Intramolecular reactions between oxidized and ground-state residues in proteins can result in significant changes in the CIDNP pattern. Among such reactions, intramolecular electron transfer (IET) from a tyrosine residue to a tryptophan indolyl radical is probably the most important. By changing the concentration of tyrosine and tryptophan radicals, the IET reaction should affect the kinetics of nuclear polarization formation for tryptophan and tyrosine protons. Previously the IET reaction has been studied in a number of peptides and proteins, mostly by using pulse radiolysis.[9−17] In D_2O IET proceeds about 3 times slower than that in H_2O, and this was attributed to an increase in the energy expended for solvent reorganization.[13]

The aim of the present paper is to present a quantitative study of the influence of IET on the formation of CIDNP in photochemical reactions of a L-TrpH-L-TyrOH dipeptide with 2,2′-dipyridyl under acidic conditions. The results obtained under the same experimental conditions for dipeptide and for an equimolar mixture of two nonlinked amino acids (*N*-acetyl tryptophan and *N*-acetyl tyrosine) are compared. The structures of the compounds under study are presented in Chart 1.

Our interest in these systems stems from results on protein folding detected by CIDNP recently published by Hore et al.[18] To shed light on the dynamic and structural changes that may be taking place during the folding process, we have applied *the time-resolved* CIDNP method instead of stationary CIDNP. To investigate the factors governing CIDNP formation in the presence of radical transformation via IET, we have performed a detailed kinetic analysis of polarization in a simple dipeptide prior to investigation of proteins. A similar investigation of hen egg white lysozyme in denatured and native states, which will be published later, allows us to suggest that the drastic difference between CIDNP patterns in the denatured and native states of a protein in steady-state experiments are partly caused by different efficiencies of IET reactions in these states.

Experimental Section

A detailed description of our TR−CIDNP apparatus has been given previously.[19] A sample, purged with argon and sealed in a standard NMR Pyrex ampule, was irradiated by a COMPEX Lambda Physik excimer laser (wavelength 308 nm, pulse energy up to 150 mJ) in the probe of an MSL-300 Bruker NMR spectrometer. TR−CIDNP experiments were carried out using the usual pulse sequence: saturation−laser pulse−evolution time−detection pulse−free induction decay. Because 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 (rf) pulse appear in the CIDNP spectra. For kinetic measurements, the rf pulse with the duration of 1 μs was used. The concentration of 2,2′-dipyridyl used in CIDNP experiments varied in the range of $(4.5-7.0) \times 10^{-4}$ M.

LFP measurements were performed using an apparatus described earlier.[20,21] All LFP and CIDNP experiments were carried out at pH = 3.8.

D_2O (Aldrich), 2,2′-dipyridyl (Aldrich), L-tryptophan-L-tyrosine and individual amino acids (Sigma) were used as received, and H_2O was doubly distilled.

Results and Discussion

LFP Measurements. In acidic solutions, both ground-state 2,2′-dipyridyl DPH^+ ($pK_a = 4.3$)[22] and its triplet $^TDPH^+$ ($pK_a = 5.8$)[8] exist in the protonated state. Under these conditions the quenching of $^TDPH^+$ by both TrpH and TyrOH proceeds via electron transfer with similar rate constants for each individual amino acid ($k_{q(TrpH)} = 4.5 \times 10^9$ M^{-1} s^{-1} and $k_{q(TyrOH)} = 3 \times 10^9$ M^{-1} s^{-1}).[6,7] Thus, at pH 3.8 one can expect generation of both tryptophan indolyl and tyrosine phenoxyl radicals as the result of quenching of triplet dipyridyl by the dipeptide:

$$DPH^+ \xrightarrow{h\nu,\mathrm{ISC}} {}^TDPH^+ \tag{1}$$

$$^TDPH^+ + TrpH - TyrOH \xrightarrow{k_{q1}} DPH^\bullet + TrpH^{+\bullet} - TyrOH \tag{2a}$$

$$^TDPH^+ + TrpH - TyrOH \xrightarrow[-H^+]{k_{q2}} DPH^\bullet + TrpH - TyrO^\bullet \tag{2b}$$

$$DPH^\bullet + H^+ \rightleftarrows DPH_2^{+\bullet} \tag{2c}$$

The cation radical of tyrosine $TyrOH^{+\bullet}$, formed by electron transfer, undergoes very fast deprotonation, whereas $DPH^\bullet$ at pH 3.8 transforms into $DPH_2^{+\bullet}$ ($pK_a = 8.5$).[8]

The rate constant of $^TDPH^+$ quenching by L-TrpH-L-TyrOH, measured by the rate of triplet absorption decay at 325 nm at different peptide concentrations, is equal to $k_q = k_{q1} + k_{q2} = (2.5 \pm 0.3) \times 10^9$ M^{-1} s^{-1}.

The reaction of IET from a tyrosine residue to the tryptophan cation radical is accompanied by fast deprotonation of $TyrOH^{+\bullet}$, followed by the reactions of radical termination:

$$TrpH^{+\bullet} - TyrOH \xrightarrow[-H^+]{k_1} TrpH - TyrO^\bullet \tag{3}$$

$$TrpH^{+\bullet} - TyrOH + DPH_2^{+\bullet} \xrightarrow{k_t} \text{products} \tag{4a}$$

$$TrpH - TyrO^\bullet + DPH_2^{+\bullet} \xrightarrow{k_t} \text{products} \tag{4b}$$

The rate constant for radical termination $k_t = (1.2 \pm 0.3) \times 10^9$ M^{-1} s^{-1} was determined by the measurement of the decay of $DPH_2^{+\bullet}$ at its absorption maximum 370 nm, using the known absorption coefficient $\epsilon = 4.5 \times 10^4$ $M^{-1}cm^{-1}$.[6]

The IET reaction in TrpH-TyrOH can be monitored either by the signal growth at 405 nm (maximum of $TyrO^\bullet$ absorption) or by the signal decay at 570 nm (maximum of $TrpH^{+\bullet}$ absorption). However, since both dipyridyl derivatives (triplet state and radical) strongly absorb at 405 nm and distort the kinetics of $TyrO^\bullet$ absorption (Figure 1), the rate constant of IET k_1 was determined from kinetic measurements at 570 nm only. The bold line in Figure 1 presents the kinetic simulations according to the reaction scheme described by eqs 2a−4b. The rate constant k_1 was determined from the best fit of calculated and experimental kinetic curves. At pH 3.8 the value of k_1, measured in H_2O, is equal to $(1.0 \pm 0.1) \times 10^6$ s^{-1}, and in deuterated water $k_1 = (6.5 \pm 0.8) \times 10^5$ s^{-1}. The observed isotope effect is in a good agreement with results observed earlier for neutral solutions.[13]

CIDNP Effects on the Mixture of Nonlinked Amino Acids. ^1H CIDNP spectra shown in the left part of Figure 2 were obtained at pH 3.8 during the irradiation of a solution containing 2,2′-dipyridyl and the equimolar mixture of *N*-acetyl derivatives

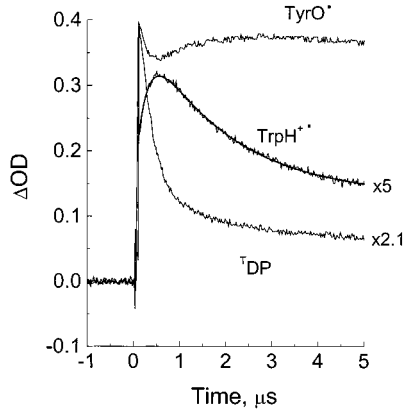


Figure 1. Kinetics of $^{T}DPH^{+}$ decay (monitored at 325 nm) and of evolution of $TyrO^{\bullet}$ (405 nm) and $TrpH^{+\bullet}$ (570 nm) radicals in H_2O, pH 3.8. Solid line: best fit obtained for the model calculation according to reactions 1−4 with the following parameters: $k_q = 2.5 \times 10^9$ M^{-1} s^{-1}, $k_1 = 6.5 \times 10^5$ s^{-1}, and $k_t = 1.2 \times 10^9$ M^{-1} s^{-1}.

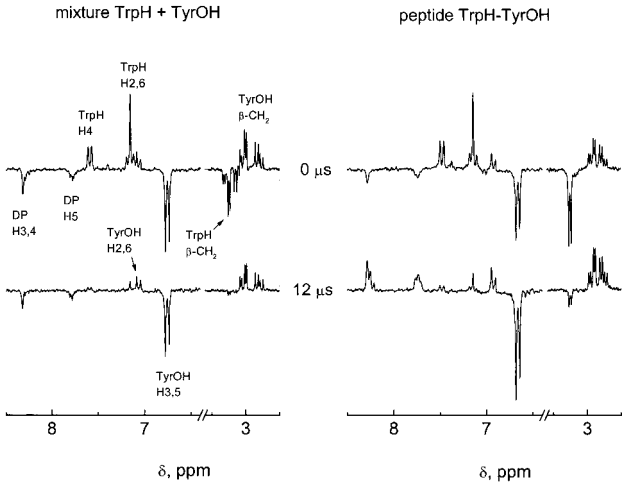


Figure 2. ^{1}H CIDNP spectra obtained during the irradiation of the solution containing 2,2′-dipyridyl and (left) 1.3 mM of *N*-acetyl tryptophan and 1.3 mM of *N*-acetyl tyrosine, (right) 1 mM of the dipeptide tryptophan-tyrosine. Time delay between the laser pulse and the rf pulse was 0 μs (top spectra) and 12 μs (bottom spectra).

of the amino acids, tryptophan and tyrosine, at different time delays between laser pulse and detecting radio frequency pulse. The assignments of the H4 and H5 signals of 2,2′-dipyridyl in our previous papers[6,7] were transposed (i.e. our initial assignment of these signals was incorrect). Under the same experimental conditions, at each delay, the CIDNP spectra obtained for DP and the mixture of amino acids appear to be a superposition of the polarization patterns of DP with individual amino acids. The phases of the CIDNP signals of TrpH and TyrOH are in agreement with Kaptein's rules.[23] The polarization of the dipyridyl protons is formed in two types of RP's with different signs of Δg: $\Delta g > 0$ for the RP with the tryptophanyl radical, and $\Delta g < 0$ in the RP with the tyrosyl radical. According to Kaptein's rules, CIDNP created in the DP protons in the RP with the tryptophanyl radical is negative (emission), whereas in the RP with the tyrosyl radical, positive polarization (enhanced absorption) is formed on the protons of dipyridyl. The resulting CIDNP for DP depends on two quantities. The first is the ratio of the enhancement factors in the two types of RPs, which is determined by the magnetic properties of the radicals. The second is the share (the ratio of the concentration of each type of RPs to the total RP concentration*)* for each type of geminate RP. This second quantity reflects the ratio of the

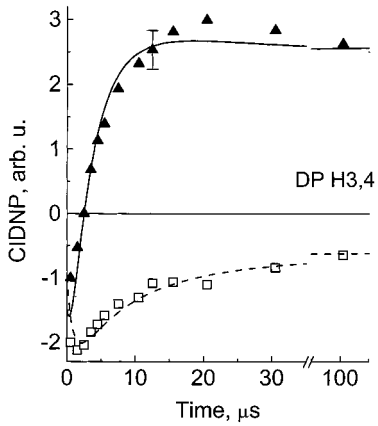


Figure 3. CIDNP kinetics for H3,4 of 2,2′-dipyridyl obtained during photoreaction with the dipeptide tryptophan-tyrosine (▲) and with a mixture of *N*-acetyl tryptophan and *N*-acetyl tyrosine (□). Concentrations were 1 mM for the dipeptide and 1.3 mM for *N*-acetyl derivatives of amino acids, pH = 3.8. Solid line: calculation as described in the text using the parameters $R_0 = 2.5 \times 10^{-4}$ M, $T_{DP} = 44$ μs, $k_t = 1.2 \times 10^9$ M^{-1} s^{-1}, $k_1 = 5.0 \times 10^5$ s^{-1}. Dashed line: calculation for the case of the mixture of *N*-acetyl tryptophan and *N*-acetyl tyrosine.

quenching rate constants of triplet excited dipyridyl by tryptophan $k_{q(TrpH)}$ and tyrosine $k_{q(TyrOH)}$. For our experimental conditions (pH = 3.8), $k_{q(TrpH)}/k_{q(TyrOH)} \approx 1.5$ (see above). The protons of DP are in emission, i.e., "tryptophan type" polarization prevails. It is seen in Figure 2 that all CIDNP signals decay in time, in complete accordance with the previous CIDNP studies of photoreaction of DP with individual amino acids.[6,7] This decay is associated with two bulk processes, which transfer the nuclear polarization of opposite phase to diamagnetic products, and result in CIDNP cancelation: (i) radical recombination and (ii) degenerate electron exchange between polarized radicals and diamagnetic molecules (see below). At pH 3.8, the latter process is important for tryptophan only.[6,7]

CIDNP Spectra in the Reaction of DP with Peptide. Dark NMR spectra of the dipeptide and the equimolar mixture of *N*-acetyl derivatives of tyrosine and tryptophan are very similar to each other except for the splitting pattern of β-CH_2 protons of tryptophan (the chemical shifts of those coincide). The absolute and relative intensities in the CIDNP spectrum obtained during the irradiation of a solution containing 2,2′-dipyridyl and the dipeptide immediately after the laser pulse (Figure 2, upper right) and in the CIDNP spectrum of the amino acids' mixture (Figure 1, upper left) are very similar. This is good evidence that under our experimental conditions (pH = 3.8), the primary photochemical processes in these two systems are the same, and the ratio of the rate constants for triplet dipyridyl quenching by both residues of peptide is similar to that for the case of free amino acids ($k_{q1}/k_{q2} \approx 1.5$ for reactions 2a and 2b).

However, the kinetic behavior of CIDNP signals in the case of a mixture of the unlinked amino acids and the covalently linked dipeptide are different (Figure 2, bottom). The signals of tyrosine in the dipeptide exhibit growth in the time interval from 0 to 12 μs, while signals of tryptophan decay in time. The most dramatic changes are observed for the protons of DP. In the reaction with the dipeptide this polarization turns from emission ("tryptophan type") to enhanced absorption ("tyrosine type"). CIDNP kinetics obtained for different protons in the reaction of DP with the mixture and the dipeptide under the same experimental conditions are shown in Figures 3−5. All of the data in these kinetic curves are obtained with the same scaling factor. The decay of the tryptophan CIDNP signal is accompanied by a growth of the tyrosine polarization. This is

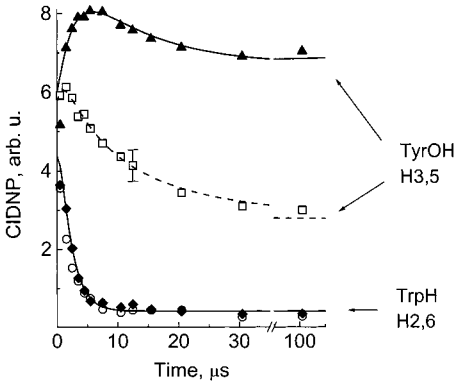


Figure 4. CIDNP kinetics for the H3,5 protons of tyrosine residue in the dipeptide (▲), H3,5 of *N*-acetyl tyrosine (□), H2,6 of tryptophan residue in peptide (♦), and H2,6 of *N*-acetyl tryptophan (○), obtained during photoreactions of the corresponding compounds with 2,2′-dipyridyl. Concentrations used were 1 mM for the dipeptide and 1.3 mM for *N*-acetyl derivatives of the individual amino acids, pH = 3.8. Solid lines: calculation as described in the text using the parameters $R_0 = 2.5 \times 10^{-4}$ M, $T_{Tyr} = 33$ μs, $T_{Trp} = 26$ μs, $k_t = 1.2 \times 10^9$ M^{-1} s^{-1}, $k_{ex} = 0$, and $k_1 = 5.0 \times 10^5$ s^{-1}. Dashed line: calculation for the case of the mixture of *N*-acetyl tryptophan and *N*-acetyl tyrosine.

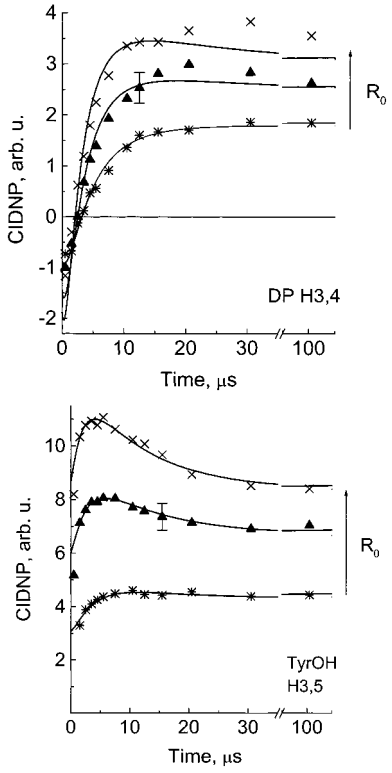


Figure 5. CIDNP kinetics for H3,4 of 2,2′-dipyridyl (a) and H3,5 of tyrosine residue (b) obtained at the initial peptide concentrations 0.5 mM (×), 1 mM (▲), and 2 mM (*). Solid lines: calculation as described in the text using the parameters $R_0 = 3.3 \times 10^{-4}$ M (upper curves), $R_0 = 2.5 \times 10^{-4}$ M (middle curves), $R_0 = 1.4 \times 10^{-4}$ M (lower curves), $T_{DP} = 44$ μs, $T_{Tyr} = 33$ μs, $k_t = 1.2 \times 10^9$ M^{-1} s^{-1}, and $k_1 = 5.0 \times 10^5$ s^{-1}.

good evidence that in the dipeptide TrpH-TyrOH, the observed CIDNP effects are determined not only by the processes unique to the individual amino acid radicals, but also by the reaction of intramolecular electron transfer from tyrosine terminal to the tryptophan radical.

In the presence of the reaction 3, the concentrations of the radicals of dipyridyl (R_{DP}), tryptophan (R_{Trp}), and tyrosine (R_{Tyr}) are each described by the following equations:

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

$$R_{Trp}(t) = \frac{\alpha R_0 e^{-k_1 t}}{1 + k_t R_0 t} \tag{6}$$

$$R_{Tyr}(t) = \frac{R_0(1 - \alpha e^{-k_1 t})}{1 + k_t R_0 t} \tag{7}$$

Here, R_0 is the initial concentration of dipyridyl radicals, or the sum of the concentrations of tryptophan and tyrosine radicals of peptide; α is the fraction of tryptophan radicals, $\alpha = k_{q1}/(k_{q1} + k_{q2})$; k_t is the bimolecular termination rate constant (here we presume that radical termination proceeds with the same rate constant for all radicals involved); and k_1 is the rate constant for the electron-transfer reaction in the dipeptide. We have neglected the fraction of geminate recombination and consider formation of radical pairs to be an instantaneous process, thus R_0 is the initial concentration of radical pairs. The concentration of tyrosine radicals increases in time from the initial value of $(1-\alpha)R_0$ to the maximum and then decays (see eq 7). This increase of the tyrosine radical concentration results in CIDNP growth from the geminate polarization to the maximum. Quantitative kinetic measurements and simulations of the CIDNP profiles are now considered.

CIDNP Kinetics. Generally, the following processes determine the CIDNP kinetics in a reversible reaction at high magnetic field. The radicals formed initially recombine partly in the cage, giving geminate polarization. In high magnetic fields CIDNP is created by the spin-sorting $S-T_0$ mechanism, and escaped radicals carry polarization equal in magnitude but opposite in phase to the geminate polarization. In radical reactions in the bulk, escape polarization is transferred to diamagnetic products, resulting in CIDNP cancelation. However, nuclear paramagnetic relaxation decreases the magnitude of escape polarization in radicals and leads to incomplete cancellation. Also, additional CIDNP can be created during random radical encounters in so-called F-pairs in the bulk. In case of a triplet precursor, the signs of geminate and F-pair polarization coincide.

Three bulk reactions lead to polarization transfer from radicals to products:

(i) termination (k_t) via back electron/hydrogen atom transfer to give the starting compounds; (ii) degenerate electron exchange ($^*RH^{+\bullet} + RH \rightarrow {}^*RH + RH^{+\bullet}$ (k_{ex})), where the asterisk denotes nuclear polarization. At pH = 3.8 only the tryptophan cation radical is involved in a degenerate electron exchange reaction with ground-state tryptophan; (iii) intramolecular electron transfer (reaction 3) in the dipeptide (k_1). It converts a tryptophanyl radical with polarized nuclei into a diamagnetic molecule. The tyrosyl radical, formed in this process, carries no nuclear polarization. However, CIDNP on the tyrosine terminal of the dipeptide is later created in the bulk via F-pairs formed with a dipyridyl radical.

Kinetic measurements were performed at three different initial concentrations of dipeptide, and this had an influence on the observed CIDNP effects in two ways. First, the initial concentration changes with a pseudo-first-order exchange rate constant k_{ex}. Second, the initial radical concentration R_0 (and the magnitude of polarization) is proportional to the ratio $(k_q[TrpH - TyrOH])/(k_d + k_q[TrpH - TyrOH])$, where k_d represents the pathways for $^TDPH^+$ decay other than quenching by the dipeptide, typically by reaction with residual oxygen.

For simulation of the CIDNP kinetics in the dipeptide we used the general approach suggested by Fischer,[24] as it has been used before for reversible radical photoreactions of DP with individual amino acids.[6−8] The processes leading to formation of CIDNP in diamagnetic products in case of the dipeptide is more complex: three types of radicals are present in the solution, and the reaction 3 converts the tryptophanyl radical of the dipeptide into the tyrosyl radical. The time evolutions of the corresponding radical concentrations are described by eqs 5−7. Thus, the system of 9 coupled differential eqs 5−13 describes the time dependence of the following: the concentrations of the radicals of dipyridyl R_{DP}, tryptophan R_{Trp}, and tyrosine R_{Tyr}; the polarization in the radicals of dipyridyl $P_{DP}{}^{R}$, tryptophan $P_{Trp}{}^{R}$, and tyrosine $P_{Tyr}{}^{R}$; the polarization in the ground-state molecules of dipyridyl P_{DP}, tryptophan P_{Trp}, and tyrosine P_{Tyr}.

$$\frac{dP_{DP}{}^{R}}{dt} = -k_t P_{DP}{}^{R}(R_{Trp} + R_{Tyr}) - k_t\beta_1 R_{DP}R_{Trp} - k_t\beta_2 R_{DP}R_{Tyr} - \frac{P_{DP}{}^{R}}{T_{DP}} \quad (8)$$

$$\frac{dP_{DP}}{dt} = k_t P_{DP}{}^{R}(R_{Trp} + R_{Tyr}) + k_t\beta_1 R_{DP}R_{Trp} + k_t\beta_2 R_{DP}R_{Tyr} \quad (9)$$

$$\frac{dP_{Trp}{}^{R}}{dt} = -k_t P_{Trp}{}^{R}R_{DP} - k_t\beta_{Trp}R_{DP}R_{Trp} - \frac{P_{Trp}{}^{R}}{T_{Trp}} - k_{ex}P_{Trp}{}^{R} - k_1 P_{Trp}{}^{R} \quad (10)$$

$$\frac{dP_{Trp}}{dt} = k_t P_{Trp}{}^{R}R_{DP} + k_t\beta_{Trp}R_{DP}R_{Trp} + k_{ex}P_{Trp}{}^{R} + k_1 P_{Trp}{}^{R} \quad (11)$$

$$\frac{dP_{Tyr}{}^{R}}{dt} = -k_t P_{Tyr}{}^{R}R_{DP} - k_t\beta_{Tyr}R_{DP}R_{Tyr} - \frac{P_{Tyr}{}^{R}}{T_{Tyr}} \quad (12)$$

$$\frac{dP_{Tyr}}{dt} = k_t P_{Tyr}{}^{R}R_{DP} + k_t\beta_{Tyr}R_{DP}R_{Tyr} \quad (13)$$

Here k_{ex} is pseudo-first-order rate constant of degenerate electron exchange, which is a product of a second-order rate constant and the dipeptide concentration; T_{DP}, T_{Trp}, and T_{Tyr} are the relaxation times of the nuclei in the respective radicals. The polarization created in the F-pairs is related to the parameter β, which is proportional to the value of geminate polarization P^G:

$$\beta = \gamma P^G/R_0 \quad (14)$$

The quantity γ is the ratio of CIDNP created in geminate versus F-pairs, and is believed to be equal to 3 in case of a triplet precursor. Equations 8−13 include different β values, depending on the type of peptide radical. To take into account the formation of CIDNP for dipyridyl in two types of RPs (with tryptophanyl and tyrosyl radicals, respectively) we used different values β_1 and β_2 in eqs 8 and 9.

Although a large number of parameters are present in eqs 5−13, many of them are known from independent measurements. The parameter α was taken to be 0.6 from the ratio of the quenching rate constants at pH = 3.8. For the parameter γ we used the value 2.8,[6] except the case of tyrosine, for which a value of $\gamma = 1.4$ was found in our previous investigation.[7] The termination rate constant k_t was taken from our optical measurements (1.2×10^9 M^{-1} s^{-1}, see above).

For the protons of tryptophan and tyrosine residues in the dipeptide, paramagnetic relaxation times were fitting parameters. The best fits were obtained using 26 μs for H2,6 of tryptophan and 33 μs for H3,5 of tyrosine. These values are approximately 1.7 times shorter than the relaxation times of the corresponding protons in the individual amino acids. This fact is in agreement with lengthening of rotational correlation time upon peptide formation, resulting from increasing molecular size in comparison with the individual amino acids.

For dipyridyl, we used the previously obtained value of $T_{DP} = 44$ μs,[6−8] and the only fitting parameter for this signal was the ratio of geminate polarization with tryptophan and tyrosine radicals, found to be −1.7. The absolute value of this parameter is higher that the estimated ratio of $k_{q1}/k_{q2} = 1.5$. This is in agreement with our qualitative conclusion that polarization formed in the radical pair with tryptophan prevails (see above), and the phase is opposite in sign to that of dipyridyl for these two pairs.

For the mixture of amino acids, calculations were performed according to the system of eqs 5−13 without taking into account IET, and with parameters taken from the previous papers.[6,7]

For CIDNP in the dipeptide, the results of simulations for 3 different initial concentrations of radicals R_0 are shown in Figures 3−5 (solid lines). The parameters for the calculations are given in the figure captions. The value k_1 that fitted all the experimental data was found to be 5×10^5 s^{-1}. This is in good agreement with our optical measurements in D_2O (see above). Variation of the dipeptide concentration up to 2 mM did not change the decay of TrpH polarization, meaning that the exchange rate constant for the dipeptide is below 10^8 M^{-1} s^{-1}. This is not surprising since this reaction occurs between a large zwitterionic molecule and a radical carrying two positive charges and one negative charge. It can be seen from our experiments with the dipeptide and the mixture of the amino acids that CIDNP kinetics for the tryptophan protons nearly coincide (Figure 4). However, for an individual tryptophan molecule, CIDNP decay is determined by degenerate electron exchange with $k_{ex} = 9 \times 10^8$ M^{-1} s^{-1}.[6]

In comparing the two methods used here, we make the following comments: For CIDNP we have high sensitivity (CIDNP enhancement factors) and high spectral resolution (up to 0.1 Hz) and time response for kinetics on the order of a dozen nanoseconds. Furthermore, CIDNP is sensitive to HFI constants and the g-factor difference in the radicals and allowing structural information about the intermediates to be obtained. The technique also allows us to study degenerate electron exchange, which is not observable by LFP. Another advantage is that we can distinguish between geminate and bulk recombination products, but in LFP only transients are detectable—there is no specific information about the geminate products. We have shown from the CIDNP data that both the Tyr and Trp moieties of the dipeptide are participating in the quenching of the triplet DP, and it would be impossible to determine this from LFP data only. In the LFP experiment the spectra are overlapped; the $TyrO^{\bullet}$ radical absorbs in the same region as the DP triplet and the radicals.

We stress that the sign change of DP CIDNP due to IET is very important and is in fact irrefutable evidence for the existence of the IET process. We did not use very short rf-pulses for CIDNP detection and we started from the radical stage in our CIDNP experiments by intention: It was undesirable to complicate the CIDNP kinetics (and its simulation) by the triplet

quenching process. For this reason more peptide was added in the CIDNP experiments.

Conclusion

Our results unambiguously show that intramolecular electron transfer between tryptophan and tyrosine residues in the dipeptide TrpH-TyrOH does affect the kinetics of CIDNP formation, whereas for a mixture of nonlinked amino acids this reaction is not important. The value of the rate constant for intramolecular electron transfer in L-tryptophan-L-tyrosine (5×10^5 s^{-1}), extracted from our model simulations of CIDNP kinetics in D_2O, is close to the value obtained from transient absorption kinetics (6.5×10^5 s^{-1}) and shows a significant kinetic isotope effect ($k_H/k_D = 1.5$). We have demonstrated that application of the time-resolved CIDNP method allows for the investigation of fast dynamic processes in peptides at the quantitative level. Due to high spectral selectivity of the method, its application to the study of dynamic processes in proteins (like migration of an electron or hydrogen deficiency) is very promising. In a forthcoming paper we will report results of a comparative study of electron transfer in native and denatured states of hen egg white lysozyme.

Acknowledgment. This work was supported by an Integrated Project Grant 65 from the Siberian Branch of Russian Academy of Science, and by the Russian Foundation for Basic Research (Projects 00-15-97450, 99-04-49879, and 99-03-33488). The research described in this publication was made possible in part by Award RC2-2208 from the CRDF and NSF (M.D.E.F., Grant CHE-9820791).

References and Notes

(1) Kaptein, R. In *NMR Spectroscopy in Molecular Biology*; Pullman, B., Ed.; Reidel: Dordrecht, 1978; pp 211−229.
(2) Stob, S.; Kaptein, R. *Photochem. Photobiol.* **1989**, *49*, 565−577.
(3) Hore, P. J.; Broadhurst, R. W. *Prog. NMR Spectrosc.* **1993**, *25*, 345−402.
(4) Muus, L. T.; Atkins, P. W.; McLauchlan, K. A.; Pedersen, J. B. *Chemically Induced Magnetic Polarization*; Reidel: Dordrecht, 1977.
(5) Salikhov, K. M.; Molin, Yu. N.; Sagdeev, R. Z.; Buchachenko, A. L. *Spin Polarization and Magnetic Field Effects in Radical Reactions*; Elsevier: Amsterdam, 1984.
(6) Tsentalovich, Yu. P.; Morozova, O. B.; Yurkovskaya, A. V.; Hore, P. J. *J. Phys. Chem. A* **1999**, *103*, 5362−5368.
(7) Tsentalovich, Yu. P.; Morozova O. B. *J. Photochem. Photobiol., A: Chemistry* **2000**, *131*, 33−40.
(8) Tsentalovich, Yu. P.; Morozova, O. B.; Yurkovskaya, A. V.; Hore, P. J.; Sagdeev, R. Z. *J. Phys. Chem. A* **2000**, *104*, 6912−6916.
(9) Land, E. J.; Pruetz, W. A. *Int. J. Radiat. Biol*. **1979**, *36*, 75−83.
(10) Pruetz, W. A.; Butler J.; Land E. J.; Swallow, A. J. *Biochem. Biophys. Res. Commun.* **1980**, *96*, 408−414.
(11) Pruetz, W. A.; Land, E. J.; Sloper, R. J. *Chem. Soc., Faraday Trans. I* **1981**, *77*, 281−292.
(12) Butler, J.; Land, E. J., Pruetz, W. A.; Swallow, A. J. *Biochim. Biophys. Acta* **1982**, *705*, 150−162.
(13) Pruetz, W. A.; Siebert, F.; Butler, J.; Land, E. J.; Menez, A.; Monteney- Garestier, T. *Biochim. Biophys. Acta* **1982**, *705*, 139−149.
(14) Butler, J.; Land, E. J., Pruetz, W. A.; Swallow, A. J. *J. Chem. Soc., Chem. Commun*. **1986**, 348−349.
(15) Faraggi, M.; DeFelippis, M. R.; Klapper, M. H. *J. Am. Chem. Soc.* **1989**, *111*, 5141−5145.
(16) Bobrowski, K.; Wierzchowski, K. L.; Holcman, J.; Ciurak, M. *Int. J. Radiat. Biol*. **1990**, *57*, 919−932.
(17) Bobrowski, K.; Poznanski, J.; Holcman, J.; Wierzchowski, K. L. *J. Phys. Chem. B* **1999**, *103*, 10316−10324.
(18) Hore, P. J.; Winder, S. L.; Roberts, C. H.; Dobson, C. M. *J. Am. Chem. Soc.* **1997**, *119*, 5049−5050.
(19) Morozova, O. B.; Yurkovskaya, A. V.; Tsentalovich, Yu. P.; Vieth, H.-M. *J. Phys. Chem. A* **1997**, *101*, 399−406.
(20) Molokov, I. F.; Tsentalovich, Yu. P.; Yurkovskaya, A. V.; Sagdeev, R. Z. *J. Photochem. Photobiol., A: Chemistry* **1997**, *110*, 159−165.
(21) Tsentalovich, Yu. P.; Kulik, L. V.; Gritsan, N. P.; Yurkovskaya, A. V. *J. Phys. Chem. A* **1998**, *102*, 7975−7980.
(22) Linnell, R. H.; Kaczmarczyk, A. *J. Phys. Chem*. **1961**, *65*, 1196−1199.
(23) Kaptein, R. *J. Chem. Soc., Chem. Commun.* **1971**, 732.
(24) Vollenweider, J.-K.; Fischer, H.; Hennig, J.; Leuschner, R. *Chem. Phys*. **1985**, *97*, 217−234.