^{1}H CIDNP study of the kinetics and mechanism of the reversible photoinduced oxidation of tryptophyl-tryptophan dipeptide in aqueous solutions

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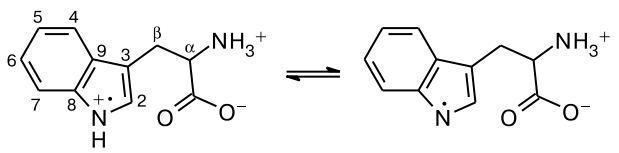
The pulsed method of chemically induced dynamic nuclear polarization (CIDNP) with the microsecond time resolution was used to study the nuclear polarization kinetics of Trp-Trp (tryptophyl-tryptophan) dipeptide protons in the photoreaction between the dipeptide and 2,2´-dipyridyl in aqueous solutions at pH from 2 to 10. The dependence of the selectivity of the reversible photoinduced oxidation of the dipeptide by 2,2´-dipyridyl in the triplet-excited state was found. When the pH of the solutions was below pK_a of the terminal amino group, polarization was observed only for the protons of the C-terminal residue. The threshold effect of protonation of the terminal amino group of the dipeptide on the selectivity of both the deactivation of the 2,2´-dipyridyl triplet state and the degenerate intermolecular electron exchange was revealed. No intramolecular electron migration in the oxidized dipeptide was detected. At the pH exceeding pK_a of the terminal amino group of the dipeptide, the quenching of the triplet excited state of 2,2´-dipyridyl is not selective. This is manifested as equal intensities of the nuclear polarization signals of the both residues of tryptophan in the ^{1}H NMR spectrum of the dipeptide containing the CIDNP signals of the geminate reaction products. The rate constant of the degenerate electron exchange and nuclear paramagnetic relaxation times of the protons of the neutral radical and radical cation of the dipeptide were determined by the numerical simulation of the nuclear polarization kinetics.

Key words: chemically induced dynamic nuclear polarization, tryptophyl-tryptophan, short-lived radicals, degenerate electron exchange, nuclear paramagnetic relaxation time.

Experimental proofs for the fact that free radicals of amino acids participate in many biochemical processes have recently been obtained. It is considered that radical reactions are responsible for the oxidative damage of proteins, which, in turn, results in a series of pathologies[1,2] and in biological aging.[2] Since the most part of proteins contain several hundreds of amino acids, the probability of their oxidative damage through radical reactions is high. Tryptophan is one of 20 amino acids present in the proteins of living organisms, and its radical products are involved in the whole series of processes. The structure of the tryptophan radicals in an aqueous solution depends on the pH (Scheme 1).

Scheme 1



$TrpH^{+\cdot}$ (pK_a = 4.3) $Trp^{\cdot}$

It was reliably established that tryptophan radicals are formed in the electron transfer reactions in a series of

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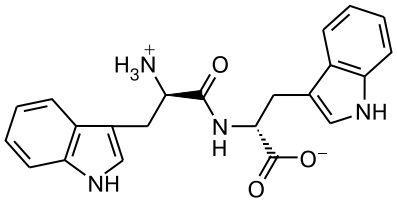
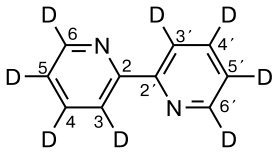
proteins, including ribonucleotide reductases,[3—5], DNA photoliases,[6—11] peroxidases,[12—20] oxidases,[21—23] and, probably, cryptochromes.[24,25] In most cases, radicals were detected by ESR spectroscopy, for example, using the ENDOR (electron-nuclear double resonance) pulsed method, because these reactive radicals can be stable only in the environment of a protein or at low temperatures usually used in ESR and ENDOR spectroscopy. The method of chemically induced dynamic nuclear polarization (CIDNP), which increases the sensitivity of NMR spectroscopy by several orders of magnitude, is an alternative approach used in this work for studying short-lived radicals of biologically important molecules in an aqueous solution. Chemically induced dynamic nuclear polarization is the nonequilibrium population of nuclear magnetic levels appeared in spin-selective reactions in which the magnetic hyperfine coupling of electron spins with nuclei plays the key role: it induces singlet-triplet transitions in the radical pair. In the NMR spectra of diamagnetic products of radical reaction, CIDNP is detected as enhanced absorption or emission signals. The advantage of the CIDNP method with time resolution which was used in this work is the possibility to easily separate contributions from geminate (pair) (duration of several nanoseconds) and bulk (microsecond and millisecond ranges) processes. The CIDNP method allows one to exactly correlate the hyperfine coupling constant (HFC) with the position of the magnetic nucleus in the radical and to determine the sign and magnitude of this constant.[26,27] At the modern level of NMR spectroscopy, this can be done for all magnetic nuclei in the radical. In ESR spectroscopy, the signal position is usually independent of the sign of the HFC constant, and isotropic substitution of reactants is often used for the assignment of HFC constants to certain nuclei in the radical structure. In addition, the rate constants of chemical reactions and nuclear relaxation times of the radicals can be determined from the nuclear polarization kinetics.

When studying the photoinduced reactions of dipeptides containing tyrosine and tryptophan,[28—30] methionine and glycine[31] by the CIDNP method, we found that the reactivity of the residues in peptide differs from that for free amino acids.[32—34] In addition, the order of connectivity of amino acids in the peptide chain and the presence/absence of a charge on the terminal amino group[35] affects the reactivity of the residues. However, no systematic investigation of this influence was carried out. For the purpose of examining the influence of the amino group charge on the oxidation of dipeptides formed from CIDNP-active amino acids, we started the systematic study of the photooxidation radical reactions in aqueous solutions. In the present work, the kinetics and mechanism of the photoinduced reactions of dipeptide tryptophyl-tryptophan (Trp-Trp) with 2,2′-dipyridyl-d_8 (DP) were systematically studied for the first time in aqueous solutions in a wide pH range.



Trp-Trp



DP

Experimental

The setup for detecting the CIDNP effects in the ^{1}H NMR spectra (hereinafter, CIDNP spectra) with time resolution was constructed on the basis of a Bruker DRX-200 NMR spectrometer (magnetic field 4.7 T, resonance frequency of protons 200 MHz). The sample in a Pyrex tube was irradiated in the NMR spectrometer with excimer laser pulses (wavelength 308 nm, energy of a pulse up to 120 mJ, pulse duration about 15 ns). The light was brought to the sample with an optical system consisting of a spherical lens, a prism, and a light guide 5 mm in diameter. For the saturation of nuclear spin states of the reactants, pulses of the broad-band homonuclear decoupler were used. After the end of the pulses, the computer of the spectrometer generated the trigger pulse to start the excimer laser, and after valuable delay τ the signal detecting radio-frequency (RF) pulse was provided. The duration of the RF pulse (1 μs) used in kinetic measurements was decreased over a duration of the $\pi/2$ pulse in order to enhance the time resolution. The free induction decay (FID) was accumulated in the standard mode, and then the experiment was repeated until the required signal to noise ratio was attained. The kinetic profile of the appearance of polarized products was obtained by the variation of delay τ.

The concentrations of reactants were selected in such a way that the characteristic quenching time of the triplet excited state of the dye would be knowingly shorter than the duration of the detecting radio-frequency pulse; *i.e.*, the product $(k_q \times C)^{-1} < 300$ ns, where k_q is the quenching rate constant of the triplet-excited dye, and C is the concentration of quenching molecules. Prior to irradiation of the sample, argon was bubbled through the solution in the ampule for 10 min to remove oxygen dissolved in water. Dipeptide L-Trp-L-Trp (Bachem), DCl, NaOD (30% solution in D_2O), and D_2O (Sigma—Aldrich) were used as received. Isotope-enriched 2,2′-dipyridyl-d_8 (C/D/N Isotopes) was used to prevent signal overlapping. The pH was varied by the addition of small amounts of DCl and NaOD. The effect of isotopic substitution on pH was not corrected.

The geometries of neutral radicals, radical cations, and radical anions of 2,2′-dipyridyl were optimized by the UB3LYP/6-31G(d) method.[36,37] The HFC constants were calculated by the same method in the EPR-II basis set taking into account the solvent (water) and using the polarized continuum model (PCM).[38] All calculations were performed using the Gaussian-03 program package.[39] The results of calculations for particles with

the open electron shell (radicals and radical ions) were not complicated by an admixture of the states with the spin $S \geq 3/2$, because for the studied particles the calculated value of $\langle S^2 \rangle$ does not exceed 0.77.

Results and Discussion

Determination of pK_a of dipeptide Trp-Trp. Since the value of pK_a for the dipeptide can differ from pK_a of individual amino acids, we recorded the ^1H NMR spectra in D_2O at pH from 2.1 to 10 in order to determine pK_a of dipeptide Trp-Trp (Fig. 1). The NMR signals at δ 7.1—7.6 (pH 2.1) are attributed to the protons of the indole rings of the dipeptide, the well resolved triplets at δ 4.58 and 4.15 (pH 2.1) are assigned to the H_α protons, and the group of signals in the region δ 3.1—3.3 (pH 2.1) belongs to the H_β protons of the dipeptide. The singlet at δ 3.29 corresponds to the methyl group of methanol, which is present as an admixture to the major substance. In our case, the protons of the β-CH_2 group are nonequivalent and form the AB system (along with the H_α protons, they form the ABX system).[29] The signals of the protons at the nitrogen atoms of the indole rings, the protons of the terminal amino group and carboxyl group, and the protons at the nitrogen atom of the peptide bond are not manifested, because these protons are exchanged with the deuterium atoms of D_2O.

To determine the acidity constants of dipeptide Trp-Trp, we used the dependences of the chemical shifts (CS) of the dipeptide protons on the pH of aqueous solutions. The ^1H NMR spectra of the dipeptide and the dependences of the CS on pH (titration curves) are shown in Figs 2 and 3. The chemical shifts of the H_α protons of the both dipeptide residues are not overlapped and depend strongly on the pH, ranging from 4.59 to 4.38 ppm with an increase

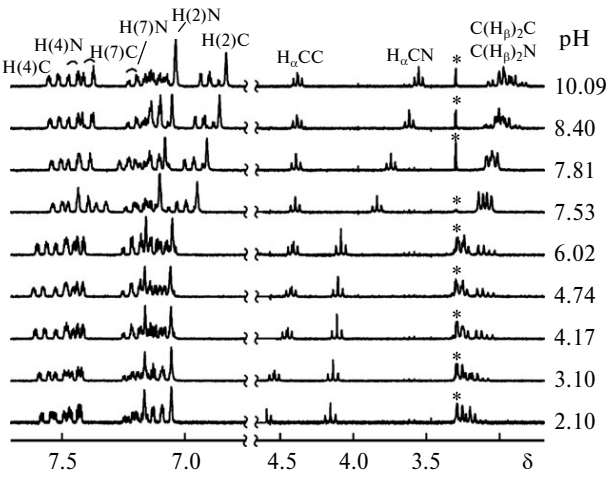


Fig. 1. ^1H NMR spectra of dipeptide Trp-Trp ($C = 5$ mol L^{-1}) in D_2O at different pH. Here and in Fig. 3, symbol * designates the signal of the Me groups of methanol.

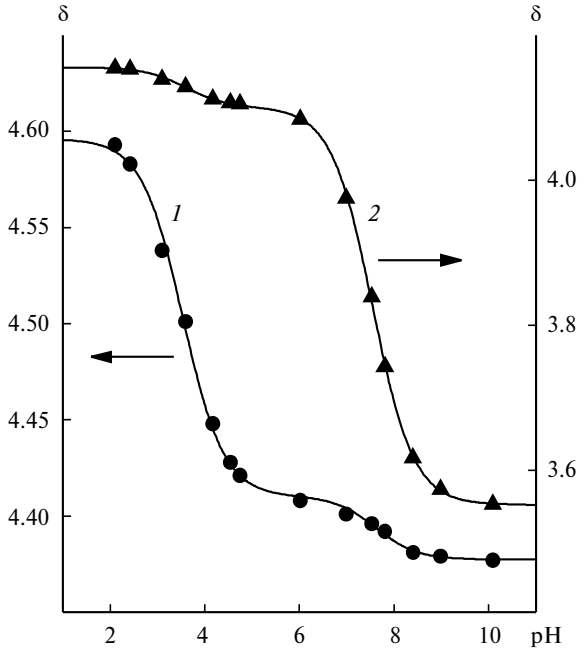


Fig. 2. Chemical shifts of the H_α protons of the C- (*1*) and N-terminal (*2*) amino acid residues of dipeptide Trp-Trp *vs* pH.

in the pH from 2.1 to 10.1 for the C-terminal residue and from 4.16 to 3.55 ppm for the N-terminal residue. The H_α proton of the C-terminal residue of the dipeptide is most sensitive to the protonation state of the carboxyl group. The H_α of the N-terminal residue of the dipeptide is most sensitive to the protonation state of the terminal amino group. The strong dependence of the CS on pH is observed for all protons of the dipeptide; however, the CS of the H_α (see Fig. 2) and H(2) protons of the both amino acid

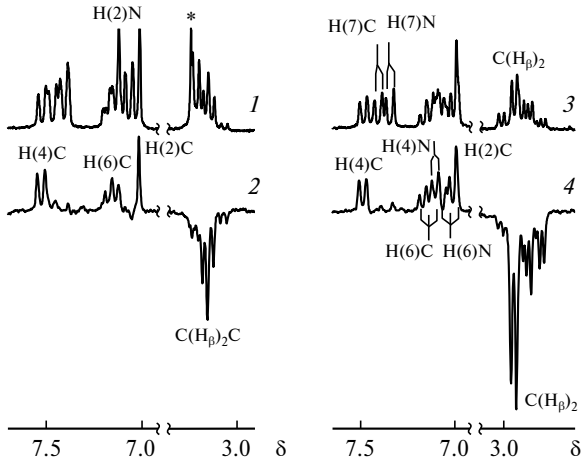


Fig. 3. ^1H NMR spectra of solutions of dipeptide Trp-Trp and DP in D_2O (*1*, *3*) and the CIDNP effects detected in the photoreactions of these solutions immediately after a laser pulse (*2*, *4*): concentration of Trp-Trp 3 mmol L^{-1} (*1*—*4*) and concentration of DP 0.6 (*1*, *2*) and 8.3 mmol L^{-1} (*3*, *4*); pH 2 (*1*, *2*) and 9.9 (*3*, *4*).

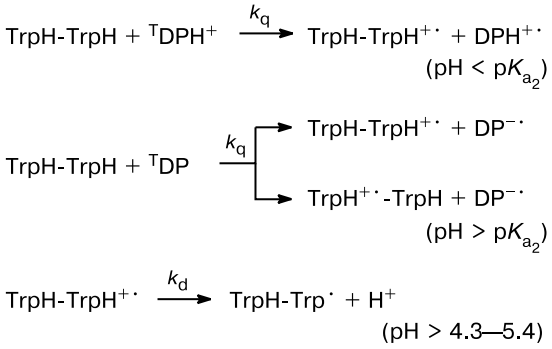
residues, whose signals were not overlapped with the signals of other protons, were chosen for simulation.

All obtained dependences of the CS of the dipeptide protons on pH were processed in combination by the least-squares method, which increased the simulation accuracy.[40,41] The following pK_a values in D_2O were obtained: $pK_{a1} = 3.5 \pm 0.1$ (carboxyl groups) and $pK_{a2} = 7.6 \pm 0.1$ (terminal amino group).

CIDNP spectra and reaction mechanism. The pH values of aqueous solutions were chosen from the obtained pK_a values for the study of the photoinduced electron transfer reaction between dipeptide Trp-Trp and dipyridyl in the triplet-excited state: 2.0, 6.6, and 9.9. At pH 2.0 the dipeptide exists as a cation with the positive charge on the nitrogen atom of the amino group, whereas at pH 6.6 it exists in the zwitterionic form with the positive charge on the nitrogen atom of the amino group and the negative charge on the carboxyl group. At pH 9.9 the dipeptide exists as an anion with the negative charge on the carboxyl group and a lone electron pair on the nitrogen atom of the amino group. The value of pK_a of the proton at the nitrogen atom of the indole moiety is higher than 14 for the diamagnetic molecule, and pK_a is 4.3 for the radical cation of tryptophan $TrpH^{+\cdot}$ and 4.3—5.4 for the tryptophan radical cation in the peptide composition.[42,43]

The protonated triplet-excited state of DP ($^TDPH^+$, pK_a 5.8)[33] in acidic solutions and the neutral triplet-excited state of DP (^TDP) in neutral and alkaline solutions are deactivated by tryptophan *via* electron transfer mechanism[32] (Scheme 2). Symbol "H" in Scheme 2 designates the hydrogen atom at the nitrogen atom in the indole ring of the C- or N-terminal residue of the dipeptide.

Scheme 2



It is impossible to distinguish radicals with radical centers on the C- and N-terminal residues of dipeptide Trp-Trp by transient absorption technique. The ^1H NMR spectrum has a high resolution (fraction of 1 Hz) and makes it possible to distinguish signals from the both residues of the dipeptide. Since the resolution typical of NMR spectroscopy is retained when recording the CIDNP effects, the contribution of each residue involved in quenching of the triplet-excited state of the dye can be determined from the geminate CIDNP spectrum. The CIDNP spectra obtained immediately after a laser pulse of the solutions containing Trp-Trp and DP at pH 2.0 and 9.9 are shown in Fig. 3 along with the corresponding NMR spectra. The signals in the CIDNP spectra were observed for the protons that were not exchanged with water and have non-zero HFC constants in intermediate radicals. Qualitatively the same CIDNP spectra as those in an acidic solution were observed at pH 6.6. The difference was in the change in the pH-dependent CS of protons of diamagnetic molecules, but this is not principal for the radical step of the reaction.

The use of the CIDNP method allowed us to establish the mechanism of dipeptide oxidation by the dye in the triplet-excited state. Although the high reactivity of Trp in peptides and proteins is known, it was found that the degree of selectivity of quenching of the triplet-excited state of DP by dipeptide Trp-Trp depends on pH. In acidic and neutral solutions, the terminal amino group of dipeptide Trp-Trp is charged and nuclear polarization was observed only for the tryptophan residue at the C-end. Therefore, in this case, the deactivation of the triplet-excited state of DP *via* electron transfer proceeds selectively and only the C-terminal dipeptide residue is involved in the reaction (see Scheme 2).

The H(2) and H(6) protons of tryptophan are chemically nonequivalent. However, in the most cases, for example, in the ^1H NMR spectrum of *N*-acetyltryptophan and, correspondingly, in the CIDNP spectrum, the signals of these protons are overlapped. In the ^1H NMR spectrum of dipeptide Trp-Trp, the signals of the H(2) and H(6) protons of the C-terminal residue did not overlap between each other but they overlapped with the signals of the H(6) and H(5) protons of the N-terminal residue. The CIDNP spectra obtained at the pH of aqueous solutions lower than pK_a of the N-terminal amino group contain no signals of the N-terminal residue. Therefore, the signals of the H(2) and H(6) protons can be integrated independently of each other.

The CIDNP spectra exhibit the signals of only the initial dipeptide and dye, indicating the high degree of reversibility of the photoinduced electron transfer. In all spectra obtained in the time range from 0 to 100 μs, only the signals from the H(2), H(4), H(6), and H_β protons of the C-terminal residue of the dipeptide remained polarized. Thus, we may conclude that, under these conditions, no intramolecular electron transfer (IET) occurs in dipeptide Trp-Trp between the tryptophan residues and the spin density at the radical step of the reaction remains only on the C-terminal residue.

At the pH higher than pK_a of the terminal amino group of dipeptide Trp-Trp, geminate nuclear polarization was observed for both the N- and C-terminal residues of the dipeptide (see Fig. 3). In this case, the both dipeptide

residues participate in the deactivation of the triplet-excite state of dipyridyl and two types of radical cations with radical centers on the C- or N-terminal residues of Trp-Trp are formed. The signals of the H(4)N and H(6)N, H(2)N and H(6)C protons overlapped and cannot be separated in the CIDNP spectrum at this pH value. However, the ratio of signals remains unchanged in time and, hence, no intramolecular electron transfer between the tryptophan residues occurs under these conditions.

CIDNP kinetics. The CIDNP kinetics is determined by contribution of polarization from the geminate product and from the recombination product in the bulk (the latter is equal by value and opposite by sign to geminate polarization) and by the contribution from polarization formed in the non-correlated radical pairs (F pairs) during their recombination. The sign of the CIDNP effect in the F pairs coincides with the sign of geminate polarization for the triplet precursor.

The products of geminate and homogeneous recombination in the reversible photochemical reactions coincide with the initial reactants. The signal descending in time is observed for CIDNP. The decrease in the CIDNP signal of the geminate products in time is caused by the compensating contribution from the products of the homogeneous combination of radicals in the bulk. Nuclear relaxation and polarization evolution in the F pairs result in the situation that the geminate polarization decreases not to the zero value. Our earlier studies of the dipeptides containing tryptophan and tyrosine[29,30] show that three processes result in the polarization transfer from the radicals to the products in solution: (1) bimolecular decay of radicals due to the backward transfer of an electron or hydrogen atom to form the initial reactants; (2) degenerate electron exchange

$$*RH^{+\cdot} + RH \longrightarrow *RH + RH^{+\cdot}$$

(asterisk marks the nuclear polarization transferred to diamagnetic molecules, and the sign of this polarization is opposite to the sign of polarization of geminate products); and (3) intramolecular electron transfer in the dipeptide.

The CIDNP kinetics of dipeptide Trp-Trp detected at pH 2.0 and 9.9 are shown in Figs 4 and 5, respectively. The normed coefficients were selected in such a way that the initial CIDNP value in the calculated curve would be equal to unity. The points in the plots correspond to the absolute values of signal intensities; *i.e.*, the polarization signs are not shown.

The fast decrease in the CIDNP signals is observed in acidic solutions, and the CIDNP kinetics of the Trp-Trp protons is determined by the reaction of degenerate electron exchange (DEE) in the pair "radical cation of dipeptide—diamagnetic molecule of dipeptide," which leads to polarization transfer from the radical cations to the diamagnetic molecules. The radical center is formed only on

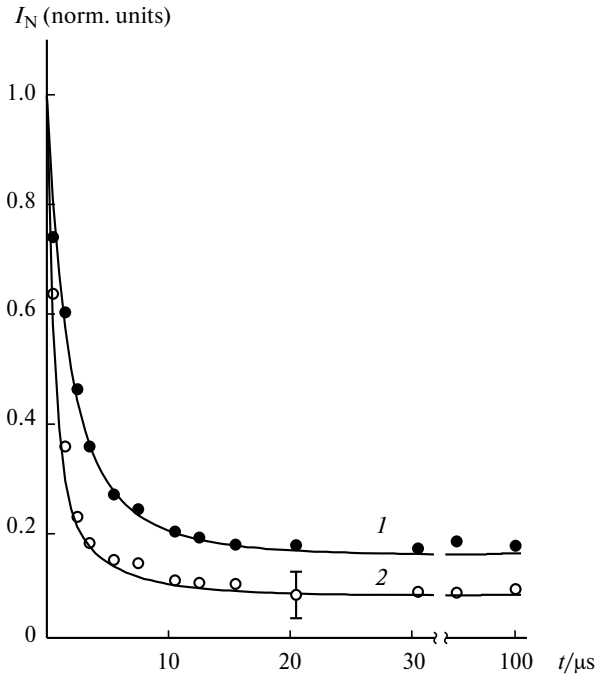


Fig. 4. CIDNP kinetics for the H(2) protons of the C-terminal residue of dipeptide Trp-Trp detected during the photolysis of DP (C = 0.6 mmol L^{-1}) with dipeptide Trp-Trp in concentrations of 2 (*1*) and 4 mmol L^{-1} (*2*) at pH 2.0. Points are experiment; lines are calculation by Eqs (1)—(3). The values of the parameters used are given in the text. Here and in Fig. 5, I_N is the normed signal intensity in the CIDNP spectrum.

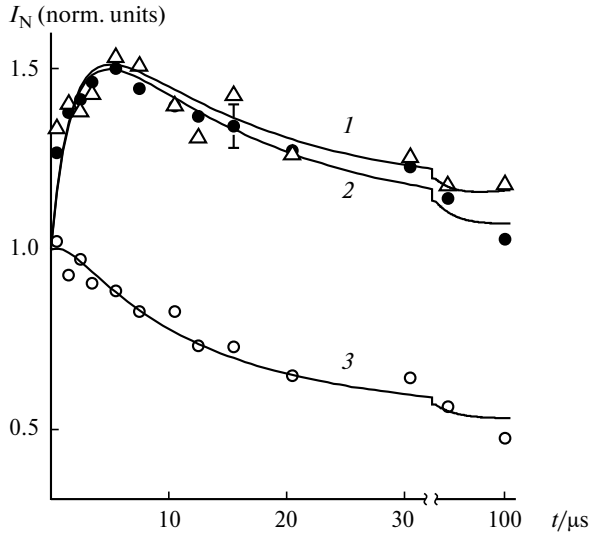


Fig. 5. CIDNP kinetics for the H(2) (*1*) and H(4) (*2*) protons of the C-terminal residue of dipeptide Trp-Trp and the H_β protons of the C- and N-terminal residues of Trp-Trp (*3*) obtained by the photolysis of DP (C = 8 mmol L^{-1}) with dipeptide Trp-Trp (C = 3 mmol L^{-1}) at pH = 9.9. Points are experiment; lines are calculation by Eqs (1)—(3). The values of the parameters used are given in the text.

the C-terminal residue of the peptide; the sign of the transferred polarization is opposite to the sign of geminate CIDNP. The degenerate electron exchange reaction involving dipeptide Trp-Trp is presented in Scheme 3.

Scheme 3

$$\text{*TrpH-TrpH}^{+\cdot} + \text{TrpH-TrpH} \xrightarrow{k_{ex}} \text{*TrpH-TrpH} + \text{TrpH-TrpH}^{+\cdot}$$

Asterisk marks particles with polarized protons.

The concentration of the reactants is much higher than that of the radicals and, hence, the DEE reaction can be considered to be the irreversible pseudo-first-order reaction with the rate constant (k_{ex}) directly proportional to the concentrations of molecules involved in the exchange (C_0): $k_{ex} = k_{ex}^{bi} C_0$, where k_{ex}^{bi} is the bimolecular rate constant of electron exchange.

In alkaline solutions, the deprotonation of the dipeptide radical cations is so fast that the degenerate electron exchange exerts no effect on the CIDNP kinetics. In both cases, the polarization first increases due to the formed polarization in the bimolecular reactions in the bulk and then decreases to the stationary value, which depends on the nuclear relaxation time in the radicals.

Since no IET was revealed and DEE proceeds selectively between the C-terminal residues of the dipeptides, the CIDNP kinetics obtained in acidic and alkaline solutions were calculated in terms of the model used previously for the description of the CIDNP kinetics in the photoreactions of free amino acids.[32,44] The model is based on the approach[45] proposed for the description of the CIDNP kinetics and includes the solution of the system of differential equations for the concentration of radicals, CIDNP in the radicals, and CIDNP in the products. The model takes into account the polarization transfer from the radical to the corresponding molecule in the bimolecular reaction of radical decay with rate constant k_t, paramagnetic relaxation with characteristic time T_1, formation of additional polarization in the radical reactions in the bulk, and polarization transfer from the radicals to the molecules in the DEE reactions with rate constant k_{ex} (the latter for the case of acidic solutions).

The concentration of the dipeptide radicals ($R(t)$) and the nuclear polarization in the dipeptide radicals ($P(R)$) and molecules ($P(Pr)$) are described by the following equations[45]:

$$R(t) = \frac{R_0}{1 + k_t R_0 t}, \qquad (1)$$

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

$$\frac{dP(Pr)}{dt} = k_t P(R) R + k_t \beta R^2 + k_{ex} C P(R). \qquad (3)$$

Equation (1) takes into account the bimolecular decay of the radicals, whose rate constant is close, in our case, to the diffusion value. The polarization formed in the radical pairs in the bulk is directly proportional to geminate polarization P^G with the proportionality coefficient $\beta = \gamma P^G / R_0$ (see Ref. 46). The γ value characterizes the CIDNP ratio in the geminate pairs and in the pairs in the bulk, and limit γ is equal to 3 in the case of the triplet precursor. Researchers usually use[32,44] $\gamma = 2.8$. The simulation gave the values of parameters $k_t R_0 = 1.8 \cdot 10^5$ s^{-1} and $k_{ex} = (4 \pm 1) \cdot 10^8$ L mol^{-1} s^{-1}, as well as the values of T_1 for the H(2), H(4), H(6), and β-CH_2 protons (Table 1). The rate constant of DEE between the radical cation of *N*-acetyltryptophan and diamagnetic molecule is $(9 \pm 1) \cdot 10^8$ L mol^{-1} s^{-1} (see Ref. 32) and that for dipeptide Trp-Tyr is $k_{ex} = (1.8 \pm 1) \cdot 10^8$ L mol^{-1} s^{-1} (see Ref. 30). In the case of the dipeptide, the decrease in the rate constant of electron exchange is caused, most likely, by steric hindrances.

When simulating the CIDNP kinetics in an alkaline solution, we took into account that no electron exchange with the diamagnetic dipeptide molecule occurs for the neutral tryptophan radicals formed due to the fast deprotonation of the radical cations. The geminate radical pair and radical pairs in the bulk differ because of the deprotonation of the dipeptide radical cation. To take into account these factors in the simulation of the kinetic data, we calculated the relative signal intensities in the CIDNP spectrum according to Adrian´s model[47] for two types of triplet radical pairs. The first pair contains the neutral radical of 2,2´-dipyridyl (DPH$^{\cdot}$) and the radical cation of the dipeptide, and the second pair contains the radical anion of 2,2´-dipyridyl (DP$^{-\cdot}$) and the neutral dipeptide radical. In the both dipeptide radicals, the electron density localized exclusively on one of the dipeptide residues: either the C-terminal or N-terminal amino acid residue. For the

Table 1. Nuclear paramagnetic relaxation times of the protons (T_1) radical cation (TrpH-TrpH$^{+\cdot}$), and neutral radical (TrpH-Trp$^{\cdot}$) of tryptophyl-tryptophan obtained by the simulation of the CIDNP kinetics in acidic and alkaline media and of the protons of the *N*-acetyltryptophan radical cation (TrpH$^{+\cdot}$)

Proton	T_1/μs		
	TrpH-TrpH$^{+\cdot}$	TrpH-Trp$^{\cdot}$	TrpH$^{+\cdot}$ [32]
H(2)	25±5	51±10	44
H(6)	33±6	*	44
H(4)	31±6	38±8	63
H_β	53±10	52±8	91

* The value of T_1 was not determined.

calculation of isotropic (A_{iso}) and anisotropic (A_{aniso}) values of the constants, we used the following magnetic resonance parameters: $g = 2.0027$ (see Ref. 48) for the radical cation of tryptophan[48] and $g = 2.0030$ (see Ref. 49) for the neutral radical and radical anion of 2,2′-dipyridyl (Tables 2 and 3).

The calculations revealed that the coefficient of enhancement of the CIDNP effect for the H(2) proton in the second radical pair is 6.5 times lower than that in the first radical pair, due to which $\gamma(H(2)) = 0.46$. The value $\gamma(H(2)) = 1$ obtained by our simulation is related, most likely, to an inaccuracy of the DFT calculations and to the fact that they were performed for the free amino acid and not for tryptophan in the peptide composition. That is why, the real value of HFC constants can somewhat differ from that presented in Table 2.

The relaxation times of the protons of the neutral dipeptide radical were determined by the simulation of the kinetic curves detected at pH 9.9 (see Table 1).

The dependence of $A^2_{aniso,i}$ (see Table 2) on $1/T_1^2$ for different protons of the neutral radical and radical cation of tryptophan is shown in Fig. 6 (see Table 1). It is seen that the dependence is linear ($R^2 = 0.995$) and starts from the point of origin. This confirms that the main mechanism of nuclear relaxation is the modulation of dipole-dipole interaction between the unpaired electron and nuclear for both the neutral radical and radical cation of tryptophan. Note that the relaxation time for the H(2) proton in the neutral radical increased substantially compared to the radical cation, which is a consequence of the higher anisotropy of the HFC tensor in the dipeptide radical cation (see Table 2 and Fig. 6).

In this work, the sensitivity of CIDNP to the reaction of degenerate electron exchange between the radical and its diamagnetic precursor was used for the identification of intermediates (neutral radicals and radical anions and cations). This reaction does not result in chemical changes and is not accompanied by a change in the absorbance of the solution and, therefore, it cannot be revealed by optical detection methods.

Thus, the kinetics and mechanism of the spin-selective reactions of photoinduced electron transfer involving dipeptide Trp-Trp in aqueous solutions at two pH values (2.0 and 9.9) were studied by the CIDNP method with time resolution. The threshold effect of the protonation of

Table 2. Isotropic (A_{iso}) and anisotropic (A_{aniso}) HFC constants of the radical cation ($TrpH^{\cdot+}$) and neutral radical ($Trp^{\cdot}$) of tryptophan calculated by the UB3LYP/EPR-II//UB3LYP/6-31G(d,p) method using the polarized continuum model

Atom	$TrpH^{\cdot+}$		$Trp^{\cdot}$	
	A_{iso}[50]/G	A^2_{aniso}	A_{iso}[50]/G	A^2_{aniso}
H(2)	−4.210	22.9	−0.53	5.82
H(1)	−4.13	32.28	—	—
H(4)	−5.043	13.62	−4.47	10.03
H(7)	−0.502	1.21	−0.35	0.94
H(6)	−4.12	13.58	−3.68	11.00
H(5)	1.24	1.03	0.79	0.86
H_β	11.89	4.01	15.42	5.19
H_β	25.44	4.88	27.87	5.7

Note. $A^2_{aniso} = {}^3\Sigma_{i=1}A^2_{aniso,i}$.

Table 3. Isotropic HFC constants (A_{iso}) of the radical cation ($DPH_2^{+\cdot}$), neutral radical ($DPH^{\cdot}$), and radical anion ($DP^{-\cdot}$) of 2,2′-dipyridyl calculated by the UB3LYP/EPR-II//B3LYP/6-31G(d) method using the polarized continuum model

Atom	A_{iso}/G		
	$DPH_2^{+\cdot}$	$DPH^{\cdot}$	$DP^{-\cdot}$
N(1)	3.09	4.07	2.08
N(1′)	3.09	2.26	2.08
H(1)	−2.85	−6.79	—
H(1′)	−2.85	—	—
H(3)	−0.19	2.16	−1.94
H(3′)	−0.19	−2.40	−1.94
H(4)	−3.60	−7.56	−0.70
H(4′)	−3.60	0.30	−0.70
H(5)	−2.36	−1.90	−5.79
H(5′)	−2.36	−4.19	−5.79
H(6)	−1.10	−2.12	0.99
H(6′)	−1.10	1.12	0.99

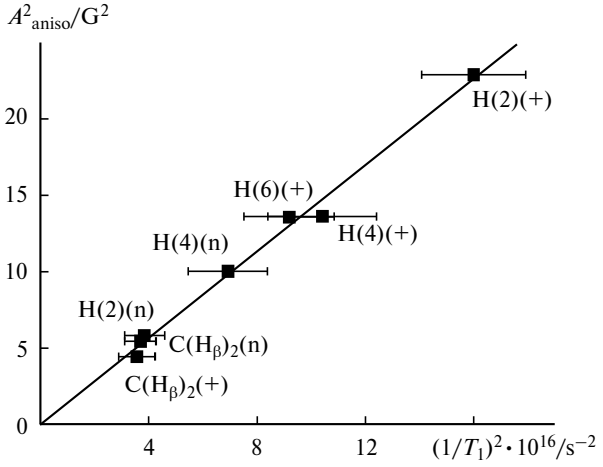


Fig. 6. Anisotropy of HFC A^2_{aniso} *vs* $1/T_1^2$, where T_1 are the nuclear paramagnetic relaxation times for the protons of the radical cation and neutral radical of Trp-Trp obtained by the simulation of the CIDNP kinetic dependences in acidic and alkaline media. Points are experiment, and the line is the linear approximation of the obtained data. Designations "+" and "n" concern the radical cation and neutral radical of the dipeptide, respectively. $A^2_{aniso} = {}^3\Sigma_{i=1}A^2_{aniso,i}$.

the amino group of dipeptide Trp-Trp on the quenching of the triplet-excited state of 2,2′-dipyridyl was revealed. At the pH of solutions lower than pK_a (pH < 7.6) of the terminal amino group, the reversible electron transfer involves only the C-terminal residue of the dipeptide. It was also established that no intramolecular electron transfer occurs from the tryptophan residue at the N-end to the tryptophan radical; however, the intermolecular degenerate electron exchange with a rate constant of $(4\pm1)\cdot10^8$ L mol^{-1} s^{-1} occurs efficiently. At the pH of the solutions higher than pK_a of the terminal amino group, the both tryptophan residues equally participate in the deactivation of the triplet state of dipyridyl and the deprotonation of the formed radical cations of tryptophan occurs in the submicrosecond time interval. The numerical simulation of the CIDNP kinetics made it possible to determine the nuclear paramagnetic relaxation times (T_1) of the protons of the neutral radicals and radical cations, which correlate well with the difference in anisotropy of the HFC tensor of these radicals calculated by the DFT method.

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