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Electron Transfer between Guanosine Radicals and Amino Acids in Aqueous Solution. II. Reduction of Guanosine Radicals by Tryptophan

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The efficiency of the chemical pathway of DNA repair is studied by time-resolved chemically induced dynamic nuclear polarization (CIDNP) using the model system containing guanosyl base radicals, and tryptophan as the electron donor. Radicals were generated photochemically by pulsed laser irradiation of a solution containing the photosensitizer 2,2′-dipyridyl, guanosine-5′-monophosphate, and *N*-acetyl tryptophan. Depending on the pH of the aqueous solution, four protonation states of the guanosyl radical are formed via electron or hydrogen atom transfer to the triplet excited dye. The rate constants of electron transfer from the amino acid to the guanosyl radical were determined by quantitative analysis of the CIDNP kinetics, which is very sensitive to the efficiency of radical reactions in the bulk, and rate constants vary from $(1.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1} for the cation and dication radicals of the nucleotide to $(1.2 \pm 0.3) \times 10^7$ M^{-1} s^{-1} for the radical in its anionic form. They were found to be higher than the corresponding values for electron transfer in the case of *N*-acetyl tyrosine as the reducing agent.

Introduction

The enzymatic DNA repair for protecting genetic information is a rather slow process,[1−3] while the electronic vacancies in DNA produced by oxidizing agents or by ionizing irradiation of the guanyl base may be refilled rather fast via electron transfer from the surrounding protein pool (in particular, from histone proteins), thus preventing the radical chemistry to evolve into pathological DNA damage.[4−9] For such a "chemical way" of DNA repair, the aromatic amino acids tryptophan and tyrosine residues were found to be the most efficient reducing agents for guanyl radicals formed in plasmid DNA under γ-irradiation. Recent reports about the influence of mild reducing agents on the enzymatic base excision repair of radiation damage in plasmid DNA demonstrated that reductive electron transfer (RET) from protein residues to guanosyl radicals formed under gamma irradiation may efficiently protect DNA bases from formation of the stable damages that are targets for enzymatic repairing activity.[4−8,10,11] The involvement of proton transfer in the reductive repair of DNA guanyl radicals by aniline compounds[6] and indoles[5] has been suggested, although the source of the proton is unclear and no direct kinetic measurements using time-resolved spectroscopic experiments have been done so far. For understanding the DNA repair mechanism by proteins, it is desirable to perform a detailed investigation of model reactions of guanosyl radicals with amino acids in a wide pH range of aqueous solution. We chose the model system consisting of guanosyl radicals generated by pulsed UV irradiation in the quenching reaction with a triplet excited dye, and an aromatic amino acid (tyrosine or tryptophan). The pulse laser photolysis has the advantages of higher time resolution and selectivity in comparison with the continuous radiochemical way of radical formation; moreover, it can be applied in combination with in situ NMR detection. An established indirect way to obtain information on radicals that are too ephemeral for electron paramagnetic resonance (EPR) detection is to employ nuclear magnetic resonance spectroscopy in combination with the phenomenon known as chemically induced dynamic nuclear polarization (CIDNP).[12,13] Observed as anomalous spectral intensities in the NMR spectra of chemically reacting systems, CIDNP arises from the spin-selective reactivity and magnetic interactions of transient spin-correlated radical pairs. Both the hyperfine interactions in the radicals and the difference in electronic *g*-value of interacting radicals drive the coherent interconversion of the singlet and triplet radical pair states. As a result, the diamagnetic products formed when the radicals recombine exhibit substantial nuclear spin polarization, whose magnitude reflects the hyperfine couplings in the radicals. A drastic increase in sensitivity and microsecond time resolution are attainable utilizing time-resolved detection of CIDNP.[14−16]

By the present paper we continue our study of the CIDNP kinetics in the course of photochemical reactions having the intermolecular electron transfer between guanosine monophosphate (GMP) radicals and amino acids as a central reaction step. In a previous paper we described the intermolecular electron transfer between GMP and tyrosine,[17] and the general approach applied for the analysis of CIDNP data. In the present study, the CIDNP kinetics in the reduction of GMP radicals by tryptophan (Trp) is considered. The guanosyl radicals were photochemically generated in the quenching reaction of the triplet excited dye 2,2′-dipyridyl (DP). The structures of 2,2′-dipyridyl and *N*-acetyl tryptophan are shown in Chart 1. Depending on the pH of the aqueous solution, the reduction of the four different guanosyl radicals shown in Scheme 1 was observed. The identification of the radicals was possible because of the high sensitivity of CIDNP to distinguish them through

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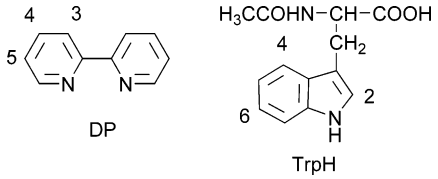
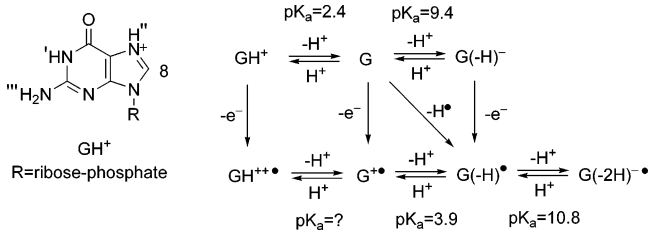
CHART 1: The Structures of 2,2′-Dipyridyl (DP) and *N*-Acetyl Tryptophan (TrpH)



SCHEME 1: Sequential Deprotonation of Guanosine-5′-monophosphate and Mechanism of Its Radical Formation[a]



[a] The protons of GMP that are sequentially released upon increasing pH are marked by an increasing number of apostrophes.

their ability or disability to participate in the degenerate electron hopping reaction with the diamagnetic molecules of GMP in the ground state. The amplitude and time evolution of CIDNP signals in this three-component system containing the dye, GMP, and the amino acid is strongly dependent on the efficiency of the RET reaction from the amino acids to the nucleotide radical.

$$GMP^{\bullet} + Trp \rightarrow GMP + Trp^{\bullet} \qquad (1)$$

The aim of our study was to explore the influence of the pH on the kinetics of the GMP radical reduction and on the structure of the participating intermediates, and to get detailed information about their reactivity.

Experimental Section

A detailed description of our setup for time-resolved CIDNP (TR-CIDNP) detection was given previously.[17,18] The samples, sealed in a standard 5 mm NMR Pyrex ampule, were irradiated by a COMPEX Lambda Physik XeCl 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 light-guide made out of 5 mm diameter polished quartz rod with its top cut at 45°.

The TR-CIDNP experiments were carried out 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 a duration of 1 μs was used for detection. To account for the spin evolution during the finite length of the RF pulse, the time scale was shifted by one-half of the RF-pulse duration, i.e., in all plots in Figures 2−5, the time of 0.5 μs corresponds to $\tau = 0$ μs, 1.5 μs stands for $\tau = 1$ μs, and so forth.

Each of the kinetic data sets recorded for the mixture of guanosine-5′-monophosphate and *N*-acetyl tryptophan was recorded using 16 samples: each sample was used to acquire four scans for each of the 13 τ-values, so that every corresponding data point in Figures 2−5 represents 64 signal accumulations. Only 32 accumulations were used to acquire the kinetic data sets for the solutions containing the amino acid and the nucleotide separately. The sample depletion after the 52 light flashes was never more than 25%. To avoid any distortion of the kinetics from this source, ascending and descending orders of the time delays were used alternately.

Guanosine-5′-monophosphate, *N*-acetyl L-tryptophan, 2,2′-dipyridyl, and D_2O were used as received from Sigma-Aldrich. The pH of the NMR samples was adjusted by addition of DCl or NaOD. No correction was made for the deuterium isotope effect on the pH.[19]

The concentrations were chosen according to the following considerations: (1) signals of the two starting compounds (Trp and GMP) should have a signal-to-noise ratio in the CIDNP spectra sufficient for quantitative analysis. (2) The lower limit of the Trp concentration is chosen to be ∼1 mM in order to be able to neglect the change of Trp concentration due to the RET reaction with GMP radicals at the estimated initial concentration of radicals below 10^{-4} M. In this case, RET can be considered a pseudo-first-order reaction. (3) The highest concentration of Trp should provide a decay time of CIDNP of GMP not shorter than the time resolution of our setup (1 μs) so that the CIDNP kinetics in the presence of RET could be treated quantitatively.

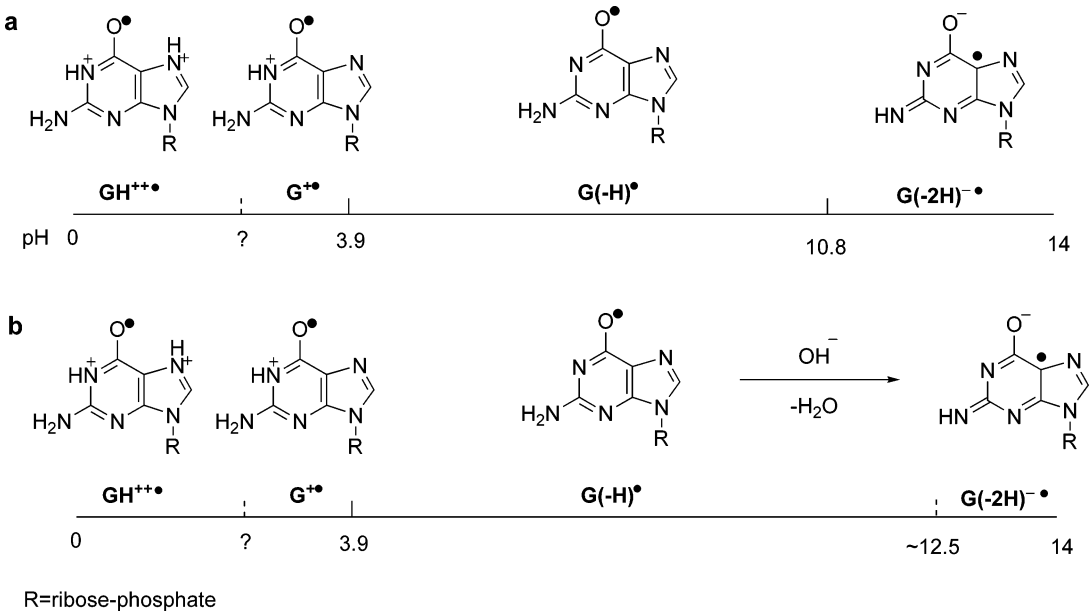
Trp, GMP, and DP are used as general abbreviations. The notations TrpH, $TrpH^{+\bullet}$ are used to indicate the protonation state of the nitrogen atom at position 1 of the corresponding molecule and radical. Tryptophan molecules with different protonation states of the carboxyl group (pK_a 2.2) are subsumed as TrpH, because the protonation state of the carboxyl group does not play any role in the electron-transfer photoreaction. When the exact protonation state of the nucleotide or its radical is mentioned, the notations from Charts 1 and 2, such as GH^+, G, and $G(-H)^-$, $GH^{++\bullet}$, and so forth, are used.

Results and Discussion

In our investigation of the reversible photocycle that includes both the oxidation and reduction of the RNA base GMP, the model system contains three compounds: GMP, the efficient photosensitizer DP, and Trp, acting as reductant of the GMP radicals. In aqueous solution, the identities of the reactive species are pH-dependent. The pK_a values of the radicals and the corresponding ground state molecules are shown in Scheme 1. Depending on the pH value of the solution, the guanine nucleotide can exist in three forms: positively charged (GH^+, p$K_a = 2.4$), neutral (G, p$K_a = 9.4$) and negatively charged ($G(-H)^-$).[20] Four different GMP radicals shown in Chart 2 were created using DP in its triplet state when varying the pH from strongly acidic to basic. The dye 2,2′-dipyridyl in aqueous solution exists as neutral dipyridyl (DP), protonated dipyridyl (DPH^+) (p$K_a = 4.3$), and double protonated dipyridyl (DPH_2^{2+}) (p$K_a = -0.2$).[21] The irradiation of DP in solution leads to the formation of its triplet state existing in either protonated ($^TDPH^+$, p$K_a = 5.8$) or neutral (^TDP) form.[22] In our previous TR-CIDNP and laser flash photolysis study, the quenching rate as a function of the pH for the reactions of triplet dipyridyl with GMP,[23] the primary step of radical formation via electron or hydrogen atom transfer, and the mechanism of CIDNP formation for the accessible range of pH were described in detail. Scheme 1 includes the mechanism of formation of the four GMP radicals from dicationic $G^{++\bullet}$ to the anionic form $G(-2H)^{-\bullet}$. The structures of GMP radicals at equilibrium condition and of GMP radicals right after the quenching reaction in the CIDNP experiment are shown in Chart 2.

At a pH lower than 2.4, the charged GMP GH^+ quenches triplet DPH^+ via electron transfer with the corresponding

CHART 2: Structure of GMP Radicals in Aqueous Solution at Different pH (a) at Equilibrium Conditions, and (b) Immediately after the Quenching Reaction in the CIDNP Experiment



quenching rate constant $k_q = 1.3 \times 10^9$ M^{-1} s^{-1}, resulting in the formation of the guanosine dication radical $GH^{++\bullet}$. Neutral triplet dipyridyl reacts with $G(-H)^-$ via electron transfer ($k_q = 1.1 \times 10^9$ M^{-1} s^{-1}) that is observed at a pH higher than 9.4, and with neutral G via hydrogen transfer ($k_q = 1.6 \times 10^8$ M^{-1} s^{-1}, 5.8 < pH < 9.4); in both reactions, the neutral radical $G(-H)^\bullet$ is formed.[23] This radical $G(-H)^\bullet$ has a pK_a of 10.8; however, at pH 11.8, the deprotonation is so slow that this radical is stable on the microsecond time scale of the CIDNP experiment.[23] We found that, in strongly basic solution (pH above 12.5), the interaction with OH^- accelerates the deprotonation of $G(-H)^\bullet$, and the anionic radical $G(-2H)^{-\bullet}$ is efficiently formed[17] (Chart 2). Assuming that $G(-2H)^{-\bullet}$ can also be formed in a proton-deficient DNA (RNA) confinement, we performed the study of the reductive interaction of the GMP radical with Trp at pH 13.3. In the case of tyrosine, the intermolecular electron transfer at this pH was found to be inefficient.[17] DP is quenched by *N*-acetyl tryptophan via electron transfer with the rate constant almost linearly decreasing from 4.7×10^9 M^{-1} s^{-1} at pH 1.9 to 2.9×10^9 M^{-1} s^{-1} at pH 13.2.[18]

The following five pH values were chosen to study the reduction of the four possible types of guanosyl radicals in Chart 2 by electron transfer from tryptophan: pH 1.3 ($GH^{++\bullet}$), pH 2.9 ($G^{+\bullet}$), pH 7.5 ($G(-H)^\bullet$), 11.0 ($G(-H)^\bullet$), and pH 13.3 ($G(-2H)^{-\bullet}$).

CIDNP Spectra. Figure 1 shows the CIDNP spectra obtained at pH 7.5 in the photoreaction of DP with GMP, DP with Trp, and DP with both species (left, right, and middle, respectively). The scaling of the three upper spectra was adjusted to have the same amplitude of the GMP H8 signal in the left pair of spectra and of the β-CH_2 signal of Trp in the right pair of spectra. The corresponding pairs of spectra taken at zero delay and 100 μs have the same scaling.

The sign Γ (positive for absorptive, negative for emissive phase) of the CIDNP signals is determined by Kaptein's rule:[24] $\Gamma = \mu \times \epsilon \times \mathrm{sgn}(\Delta g) \times \mathrm{sgn}(A)$, where $\mu = +1$ stands for a triplet precursor and $\mu = -1$ for a singlet precursor, $\epsilon = +1$ stands for geminate and $\epsilon = -1$ for escaped polarization, and $\mathrm{sgn}(\Delta g)$ and $\mathrm{sgn}(A)$ are the signs of the difference in g values and of the HFI constant of the protons under question, respectively. The ratio of CIDNP intensities of the protons belonging to the same product is usually proportional to the ratio of the HFI constants for these protons in the precursor radicals.[25] In the geminate reaction with the triplet precursor DP, enhanced absorption for H2, H6, H4 and emission for the β-CH_2 protons is formed for Trp, and emission of H8 of GMP. The protons of the photosensitizer, 2,2′-dipyridyl, acquire polarization of opposite signs in the reactions with GMP and Trp radicals: positive polarization is formed for H3,4 and H5

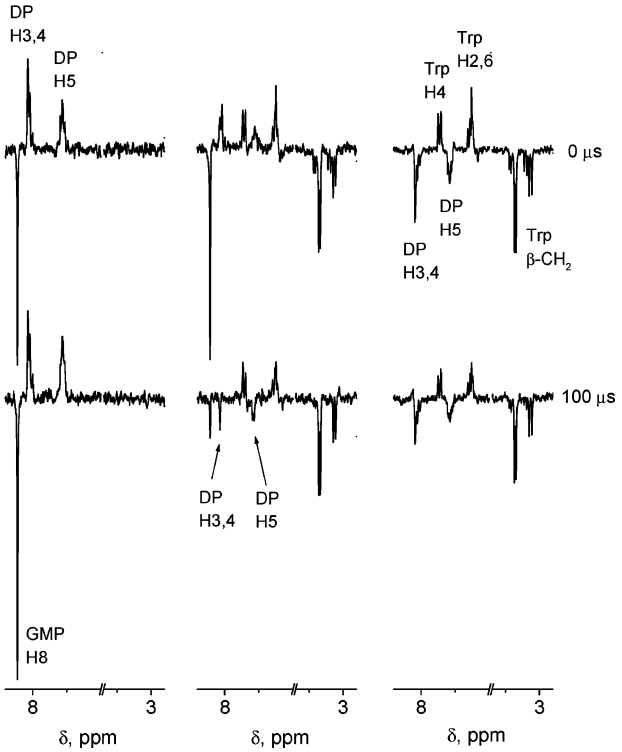


Figure 1. ^{1}H CIDNP spectra obtained in photoreactions of 2,2′-dipyridyl with 2.0×10^{-2} M guanosine-5′-monophosphate (left), 2.5×10^{-3} M *N*-acetyl tryptophan (right), and 2.0×10^{-2} M guanosine-5′-monophosphate plus 1.3×10^{-3} M *N*-acetyl tryptophan (middle). Upper spectra were taken immediately after the laser pulse, lower spectra were taken with a delay of 100 μs after the laser pulse.

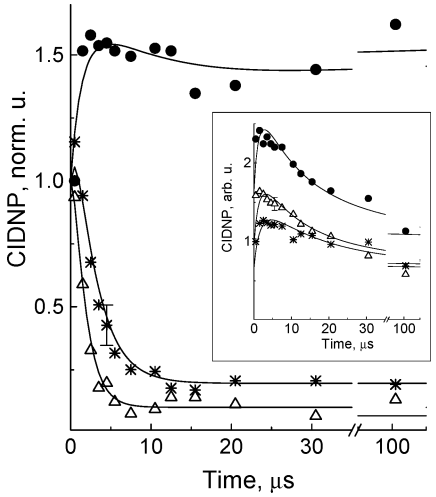


Figure 2. CIDNP kinetics for H8 of guanosine-5′-monophosphate and for β-CH_2 of *N*-acetyl tryptophan (insert) obtained at pH 7.5 in photoreactions of 2,2′-dipyridyl with 2.0×10^{-2} M guanosine-5′-monophosphate (●), with 2.5×10^{-3} M *N*-acetyl tryptophan (● in the inset), with 2.0×10^{-2} M guanosine-5′-monophosphate plus 2.5×10^{-3} M *N*-acetyl tryptophan (△), and with 2.0×10^{-2} M guanosine-5′-monophosphate plus 1.3×10^{-2} M *N*-acetyl tryptophan (∗). Lines: simulations according to the procedure described in the text with the parameters given in Tables 1 and 2.

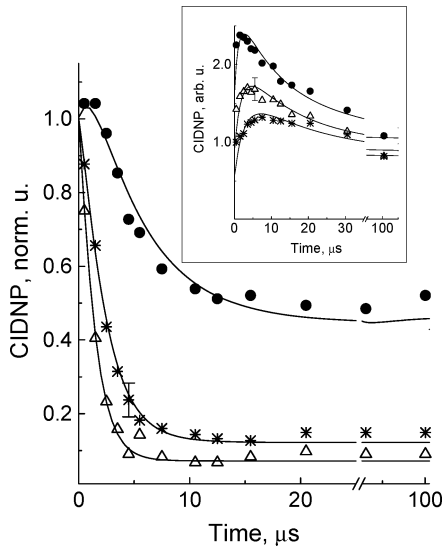


Figure 3. CIDNP kinetics of H8 of guanosine-5′-monophosphate and of β-CH_2 of *N*-acetyl tryptophan (inset) obtained at pH 11.0 in photoreactions of 2,2′-dipyridyl with 6.5×10^{-3} M guanosine-5′-monophosphate (●), with 2.5×10^{-3} M *N*-acetyl tryptophan (● in the inset), with 6.5×10^{-3} M guanosine-5′-monophosphate plus 2.5×10^{-3} M *N*-acetyl tryptophan (△), and with 6.5×10^{-3} M guanosine-5′-monophosphate plus 1.3×10^{-3} M *N*-acetyl tryptophan (∗). Lines: simulations according to the procedure described in the text with the parameters given in Tables 1 and 2.

of DP in the radical pair with a GMP radical (see two left spectra), while negative polarization results from the reaction of DP and Trp radicals (see two spectra on the right), reflecting the condition $g(\mathrm{Trp}^{\bullet}) < g(\mathrm{DP}^{\bullet}) < g(\mathrm{GMP}^{\bullet})$. The resulting CIDNP sign of the DP protons in the system, where the DP radicals are created in reactions with both GMP and Trp, depends on two factors: the first factor is the relative number of radical pairs of each type (which is a function of the relative concentrations of GMP and Trp radicals); the second factor is the ratio of CIDNP enhancement for the protons of DP in the

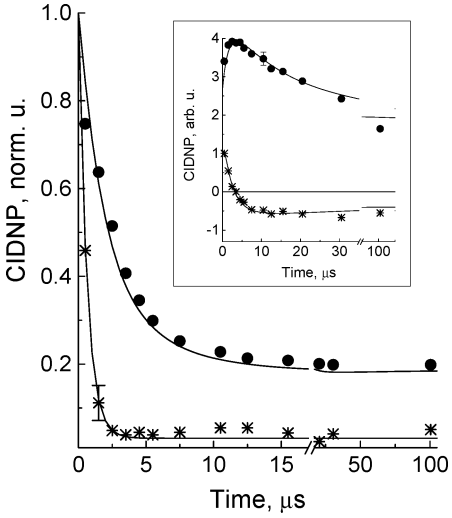


Figure 4. CIDNP kinetics of H8 of guanosine-5′-monophosphate and H3,4 of 2,2′-dipyridyl (inset) obtained at pH 1.3 in photoreactions of 2,2′-dipyridyl with 6.4×10^{-3} M guanosine-5′-monophosphate (●), and with 6.5×10^{-3} M guanosine-5′-monophosphate plus 1.3×10^{-3} M *N*-acetyl tryptophan (∗). Lines: simulations according to the procedure described in the text with the parameters given in Tables 1 and 2.

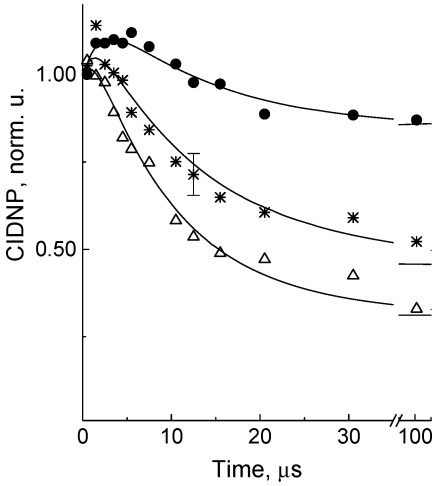


Figure 5. CIDNP kinetics of the proton H8 of guanosine-5′-monophosphate obtained at pH 13.3 in photoreactions of 2,2′-dipyridyl with 6.5×10^{-3} M guanosine-5′-monophosphate (●), with 6.5×10^{-3} M guanosine-5′-monophosphate plus 2.5×10^{-3} M *N*-acetyl tryptophan (∗), and with 6.5×10^{-3} M guanosine-5′-monophosphate plus 5.0×10^{-3} M *N*-acetyl tryptophan (△). Lines: simulations according to the procedure described in the text with the parameters given in Tables 1 and 2.

TABLE 1: Guanosyl Radicals Participating in the RET from TrpH and Rate Constants of the RET Reactions, k_e

pH	guanosine radical	k_e, $M^{-1}s^{-1}$
1.3	$GH^{++\bullet}$	
2.9	$G^{+\bullet}$	$(1.0 \pm 0.3) \times 10^9$
7.5		
11.0	$G(-H)^{\bullet}$	$(2.6 \pm 0.4) \times 10^8$
13.3	$G(-2H)^{-\bullet}$	$(1.2 \pm 0.3) \times 10^7$

two types of radical pairs with GMP and Trp radicals, respectively. The initially positive polarization observed for DP protons reflects the predominance of CIDNP generated in the pairs with GMP radicals (Figure 1, upper spectrum in the middle). As the relative concentration of Trp radicals increases

TABLE 2: Parameters[a] Used in the Simulations of the CIDNP Kinetics in Figures 2−5 According to Eqs 6−10

figure (pH)	C_{GMP}, mM	C_{Trp}, mM	fitting to symbol	k_1/k_2	$R_0k_1 \times 10^{-5}$, $M^{-1}s^{-1}$	α	$k_{ex(GMP)}$, $M^{-1}s^{-1}$	$T_{1(GMP)}$, μs	γ_{GMP}
2 (7.5)	20	2.5	Δ	0.8	3.4	0.31	0[b]	20[b]	2.8[b]
		1.3	*		3.4	0.56			
		0	●	n/a	2.6	1			
	0	2.5	● inset		2.6[a]	0			
3 (11.3)	6.5	2.5	Δ	0.6	2.0	0.42	4.0×10^{7}[b]		
		1.3	*			0.65			
		0	●	n/a		1			
	0	2.5	● inset		2.0[c]	0			
4 (1.3)	6.5	1.3	*	0.8	2.6	0.58	1.3×10^{8}[b]		
		0	●	n/a		1			
5 (13.3)	6.5	5.0	Δ	0.6	2.0	0.58	0	38[d]	1.6[d]
		2.5	*			0.47			
		0	●	n/a	1.7	1			

[a] Other parameters not listed in Table 2 are $T_{1(Trp)} = 196$ μs, $T_{1(DP)} = 100$ μs, $\gamma_{Trp} = 2.8$, and $\gamma_{DP} = 2.8$; the ratio of geminate polarization of DP, formed in a pair with a tryptophanyl and in a pair with a guanosyl radical $[P^{G1}/(\alpha R_0)]/[P^{G2}/(1-\alpha)]$ is −0.37. [b] From ref 23. [c] R_0k_2 for the kinetics of Trp with DP at $C_{GMP} = 0$ [d] From ref 17.

due to the RET reaction, the sign of DP polarization is inverted (Figure 1, bottom spectrum in the middle). A similar change in CIDNP sign but from emission to enhanced absorption was observed earlier in photoreactions of DP with the peptide tryptophan−tyrosine, when intramolecular electron-transfer takes place in the oxidized form of the peptide.[16,26,27]

The mechanism of CIDNP formation in reversible photochemical reactions has been described in a number of papers,[16,28−30] and we will only briefly repeat it here. The CIDNP kinetics in cyclic photochemical reactions, when the initial compounds and products are the same species, is determined in total by four main processes: (i) the fast rising geminate CIDNP; (ii) its compensation by the slower transfer of polarization from the escaped radicals to the diamagnetic products in bulk reactions; (iii) the loss of polarization in the radicals caused by paramagnetic nuclear relaxation, making the cancellation of the geminate CIDNP incomplete; therefore, the degree of cancellation depends on the nuclear relaxation time with respect to the radical lifetime; and (iv) additional CIDNP created in the collision of uncorrelated free radicals (F-pairs). Details of the CIDNP kinetics at each of the five pH values chosen to detect RET from tryptophan to all four possible radicals of GMP are considered below. The CIDNP signals of the β-CH_2 protons of Trp were used in our model simulations, since these protons are characterized by the highest signal-to-noise ratio and were the most convenient for the analysis.

pH 7.5. Because of the large difference in quenching rate constants for GMP ($k_{q(GMP)} = 1.6 \times 10^8$ M^{-1} s^{-1}) and Trp ($k_{q(Trp)} = 3.5 \times 10^9$ M^{-1} s^{-1}) concentrations of the starting compounds differing by about 1 order of magnitude were used. In our experiments with the systems containing only two reactants, DP/GMP and DP/Trp, the CIDNP kinetics was in accordance with previous studies.[18,23] To make the presentation of the changes in decay rate more convenient, the CIDNP kinetics of GMP in Figure 2 is scaled to normalized units by setting the initial value in the simulation to unity. For tryptophan, the variation of the CIDNP with respect to the geminate polarization is determined not only by the RET rate, but also by the relative concentration of tryptophanyl radicals with respect to guanosyl radicals. The CIDNP data for Trp were scaled in the following way: the first point in the kinetics with the lowest concentration of Trp was taken as unity, the other points were rescaled accordingly.

Upon the addition of Trp, a fast decay of the CIDNP of GMP was observed. Increasing the Trp concentration accelerates the decay rate for GMP (Figure 2). This fast decay of polarization detected for diamagnetic guanosine occurs because the G(−H)• radicals that escaped from geminate recombination carry polarization that has the same initial value but is opposite in sign to that of the geminate product. In the absence of tryptophan, efficient paramagnetic nuclear relaxation in G(−H)• with $T_1 = 16$ μs[23] destroys the nuclear polarization in the escaped radicals during their lifetime, determined by the second-order termination rate under our experimental conditions, thus no cancellation is detected.

For Trp, an increase of the stationary CIDNP value with respect to the geminate one is observed in the presence of GMP (Figure 2, inset). In general, the main peculiarities of the kinetics accompanied by RET reaction were the same as those reported for the case of *N*-acetyl tyrosine. The CIDNP kinetic data for the system GMP plus Trp are treated in a way analogous to those for GMP plus Tyr.[17] The rate constant for the RET from Trp to the G(−H)• radical extracted from the simulations is $(2.6 \pm 0.4) \times 10^8$ M^{-1} s^{-1}.

The CIDNP kinetics of DP was not analyzed quantitatively, since, at this pH, the neutral radical of DP is slowly protonated, and this process changes the spin density distribution in the DP radicals, thus preventing a quantitative description of CIDNP kinetics of DP. Since the g-factor and the HFI constants of $DPH^{+\bullet}$ agree closely with those of the neutral DP•, one can neglect the influence of the protonation on the CIDNP of other radicals that are present in the solution.

pH 11.0. At this pH, the radical of guanosine present in the solution is in its neutral form G(−H)•, as it is at pH 7.5. To check whether the RET rate constant is the same at pH 7.5 and 11.0, despite the different reaction pathways in the quenching reaction with triplet dipyridyl leading to the formation of G(−H)•, we performed measurements of the CIDNP kinetics at the same two concentrations 2.5×10^{-3} M and 1.3×10^{-3} M of *N*-acetyl tryptophan, together with 6.5×10^{-3} M guanosine-5′-monophosphate (Figure 3). The difference between the two sets of kinetic traces results from the fact that at pH 11.0 the GMP radical is involved in the degenerate electron exchange reaction with its parent molecule:

$$*G(-H)^{\bullet} + G(-H) \xrightleftharpoons{k_{ex(GMP)}} *G(-H) + G(-H)^{\bullet} \quad (2)$$

Here, the asterisk denotes nuclear polarization. Since the concentration of the starting compound is much higher than that

of the radicals produced in our experiment, reaction 2 could be treated as a pseudo-first order one, and its reversibility could be neglected. This reaction leads to an additional decay of polarization with the rate proportional to $k_{ex(GMP)} = 4.0 \times 10^7$ M^{-1} s^{-1} as it was found in ref 23.[23]

The addition of Trp accelerates the decay of GMP signals. Model simulations of the kinetics give the best fit for the RET rate constant $k_e = 2.6 \times 10^8$ M^{-1} s^{-1} coinciding within experimental error with that obtained at pH 7.5.

pH 1.3 and 2.9. In the acidic pH regime, the CIDNP kinetics of GMP in the reaction with DP is mainly determined by the efficient process of degenerate electron exchange, which at pH 1.3 is:[23]

$$*GH^{++\bullet} + GH^{+} \overset{k_{ex(GMP)}}{\rightleftharpoons} *GH^{+} + GH^{++\bullet} \quad (3)$$

with $k_{ex(GMP)} = 1.3 \times 10^8$ M^{-1} s^{-1} leading to a fast decay of the GMP signal in time. The decay of the signal is accelerated by RET (reaction 1) to the dicationic guanosyl radical $GH^{++\bullet}$ when tryptophan is added.

The CIDNP kinetics data obtained at pH 1.3 are shown in Figure 4. At this pH value, which is below the $pK_a = 4.3$ of the tryptophan radical, the kinetics of nuclear polarization for the Trp protons is also determined by the degenerate electron exchange reaction. Its rate constant as obtained earlier is high ($k_{ex(Trp)} = 9 \times 10^8$ M^{-1} s^{-1}),[18] providing a fast decay of nuclear polarization, even when GMP radicals are present in the solution in high concentration, serving as an additional source of Trp radicals. Under such efficient cancellation of polarization, the CIDNP kinetics of Trp is too fast for a quantitative analysis. Instead, the nuclear polarization of DP with its change in sign (Figure 4, inset) was simulated in the same way it was done before when the photoreaction of DP with the peptide tryptophan−tyrosine was studied.[16,26,27]

DPH radicals of the total initial concentration R_0 are formed in the following two reactions with the shares of α and $(1 - \alpha)$ for reaction 4 and reaction 5, respectively:

$$^{T}DPH^{+} + GH \xrightarrow{k_{q(GMP)}} DPH^{\bullet} + GH^{+\bullet} \quad (4)$$

$$^{T}DPH^{+} + TrpH \xrightarrow{k_{q(Trp)}} DPH^{\bullet} + TrpH^{+\bullet} \quad (5)$$

where $\alpha = k_{q(GMP)}/[k_{q(GMP)}+k_{q(Trp)}]$.

The initial concentration of GMP radicals is αR_0, while $(1 - \alpha)R_0$ is that of Trp radicals.

In the presence of the RET reaction 1, the radical concentrations are described by eqs 6−8:

$$\frac{dR_{DP}}{dt} = -k_1 R_{DP} R_{GMP} - k_2 R_{DP} R_{Trp} \quad (6)$$

$$\frac{dR_{GMP}}{dt} = -k_1 R_{DP} R_{GMP} - k'_e R_{GMP} \quad (7)$$

$$\frac{dR_{Trp}}{dt} = -k_2 R_{DP} R_{Trp} + k'_e R_{GMP} \quad (8)$$

Two types of radical pairs are considered, with partner radicals originating from dipyridyl/guanosine and dipyridyl/tryptophan. In eqs 6−10 and below, the notations 1 and 2 stand for the pairs of radicals of dipyridyl/guanosine and dipyridyl/tryptophan, respectively, with k_1 and k_2 as the corresponding second-order termination rate constants. The RET between tryptophan and GMP radicals is considered a pseudo-first-order reaction with the monomolecular rate constant proportional to the concentration of tryptophan: $k'_e = k_e C_{Trp}$. In eqs 6−8, we neglected the small fraction of geminate recombination with the triplet precursor, and considered the formation of radicals to be instantaneous.

The polarization in the dipyridyl radical (P^R) and in the diamagnetic ground state dipyridyl (P) is written as follows:

$$\frac{dP^R}{dt} = -k_1 P^R R_{GMP} - k_2 P^R R_{Trp} - k_1\beta_1 R_{DP} R_{GMP} - k_2\beta_2 R_{DP} R_{Trp} - \frac{P^R}{T_1} \quad (9)$$

$$\frac{dP}{dt} = k_1 P^R R_{GMP} + k_2 P^R R_{Trp} + k_1\beta_1 R_{DP} R_{GMP} + k_2\beta_2 R_{DP} R_{Trp} \quad (10)$$

The first and the second terms in each equation describe the polarization transfer from the radical to the ground-state molecule in the termination reaction with GMP and Trp radicals, respectively. The third and the fourth terms refer to the formation of polarization in radical pairs in the bulk, i.e., in F-pairs. Also, in eq 9, decay of nuclear polarization in the radical due to paramagnetic relaxation with the time T_1 is taken into account. The polarization, created in F-pairs is related by the parameter β 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 is taken be equal to 3 in the case of a triplet precursor. 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 9 and 10: $\beta_1 = \gamma P^{G1}/(\alpha R_0)$, $\beta_2 = \gamma P^{G2}/((1 - \alpha)R_0)$. In accordance with the spin-sorting nature of the $S-T_0$ mechanism of CIDNP formation at high magnetic field, the initial conditions are $P(0) = -P^R(0) = P^{G1} + P^{G2}$, where P^{G1} and P^{G2} denote the geminate polarization of DP formed in pairs 1 and 2, respectively.

The fitting parameters to simulate the kinetics, besides the scaling factors, were k_e, R_0k_1, k_1/k_2, and, additionally for DP, the ratio P^{G1}/P^{G2}. These parameters, together with those known from previous measurements (rate constants of degenerate exchange and paramagnetic nuclear relaxation times) are listed in Table 1. The RET rate constant extracted from the simulations is $k_e = (1.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1}.

For studying the electron transfer from tryptophan to the monocationic radicals $G^{+\bullet}$, we chose the pH 2.9, as it was done with tyrosine,[23] because we showed that, at this pH, the share of $G^{+\bullet}$ radicals resulting from the quenching of triplet $^{T}DPH^{+}$ is ~0.86. At this pH, the CIDNP kinetics of GMP is determined by the degenerate electron exchange reaction between $G^{+\bullet}$ and G with the rate constant $k_{ex(GMP)} = (3.0 \pm 0.5) \times 10^8$ $M^{-1}s^{-1}$,[23] being more than two times higher than that for the exchange between the dicationic radical $G^{++\bullet}$ and G^{+}. The CIDNP kinetics of GMP and DP obtained is very similar to that obtained at pH 1.3 (not shown). The only difference is a faster decay of nuclear polarization of GMP at pH 2.9, caused by the higher rate constant of degenerate electron exchange.[17] The rate constant of RET, $k_e = (1.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1}, is the same as that at pH 1.3.

pH 13.3. In strongly basic solution, the anionic form of guanosyl radicals, $G(-2H)^{-\bullet}$ is not formed in the quenching reaction with triplet DP directly, but as a result of interaction of the neutral radical $G(-H)^{\bullet}$ with OH^{-}, which accelerates the deprotonation of the neutral radical. We found the following three pieces of evidence allowing us to assign the escaped radical to the anionic radical $G(-2H)^{-\bullet}$. The first is the absence of the CIDNP decay peculiar to "exchange type" kinetics (Figure 5,

circles). Second, the small value of the parameter $\gamma = 1.6$,[17] as found instead of 3, reflects a change in the electron spin density distribution in the escape radical as compared to the geminate G(−H)•. Third, it is found that the detected radical has a much longer paramagnetic nuclear relaxation time $T_1 = 38$ μs in comparison with $T_1 = 20$ μs for G(−H)•.[23]

A noticeable influence of the addition of tryptophan on the CIDNP of H8 was detected at a higher concentration of Trp and is shown in Figure 5. It indicates a lower efficiency of RET from the amino acid to the nucleotide radical. The value of $k_e = (1.2 \pm 0.4) \times 10^7$ M^{-1} s^{-1} is the lowest in the whole pH region studied.

The results are summarized in Table 1. It is noteworthy that all corresponding RET rate constants are higher in the case of *N*-acetyl tryptophan than for *N*-acetyl tyrosine.

Conclusion

The reaction systems chosen as a prototype of chemical repair of DNA by fast refilling of the electron vacancy in the nucleic acid from surrounding proteins undergo the reversible photocycle that includes both the oxidation and reduction of the RNA base GMP under pulsed UV irradiation in the presence of the amino acids tryptophan and tyrosine. A great advantage of the TR-CIDNP method as applied here is that it selectively detects only radical pairs that are produced by a single laser pulse of a few nanoseconds duration, thus allowing one to detect and distinguish the contribution from geminate processes and to perform a detailed kinetic analysis of the subsequent radical reactions in the bulk.

The kinetic data for the RET between photochemically generated GMP radicals and *N*-acetyl tryptophan presented here, and for GMP radicals and tyrosine reported in our recent study,[17] demonstrate that, for both amino acids, the reaction rate constants are dependent on the protonation state of the reacting species. The detailed kinetic investigation of four forms of elusive radical intermediates of guanosine monophosphate subject to the RET from the amino acids in aqueous solution at ambient temperature was possible because of the very strong enhancement of NMR signals by CIDNP in the reaction of the triplet dye DP with both GMP and each of the two amino acids. For both amino acids, our data show that the different forms of GMP radicals, namely, $GH^{++\bullet}$ (pH 1.3), $G^{+\bullet}$ (pH 2.9), G(−H)• (pH 7.5, 11.0, 11.3), and $G(-2H)^{-\bullet}$ (pH 13.3), are characterized by their remarkably different oxidative property with rate constants changing from $(1.0 \pm 0.3) \times 10^9$ M^{-1} s^{-1}, as obtained for $G^{+\bullet}$ and $GH^{++\bullet}$ with tryptophan, to a value of less than 6.0×10^6 M^{-1} s^{-1} for the reduction of $G(-2H)^{-\bullet}$ by tyrosine. The common feature for RET is that the reaction proceeds with much higher efficiency in an acidic environment as compared to neutral or basic conditions. For tryptophan, the reaction proceeds with an efficiency that, at low pH, is about a factor of 2 higher than that for tyrosine, and is much higher at neutral and basic conditions.

From our previous studies it is known that *N*-acetyl methionine[31] and *N*-acetyl histidine[22] also exhibit strong CIDNP effects. However, no significant influence of them on the GMP signals that may be attributed to RET was detected in the range of amino acid concentration up to 20 mM in triple mixtures containing DP as photosensitizer. Because of the limited sensitivity of our TR-CIDNP apparatus, we can only give an upper limit for the rate constant of the RET from these amino acids, which is $\sim 10^6$ M^{-1} s^{-1}. The rate constant data for the reduction of guanosyl radicals in plasmid DNA by methionine derivatives is 10^5 M^{-1} s^{-1} and no reduction was found for histidine derivatives.[8]

Taking into account that the intramolecular electron transfer from tyrosine to oxidized tryptophan residue is expected to be rather efficient according to several experimental pieces of evidence reported in literature,[27] we anticipate the following scenario of sequential radical stages for "chemical repair" of DNA damages by a protein: at the first stage after light- or radiation-induced damage of any base in nucleic acids, the intramolecular electron hopping through DNA will shift the electron vacancy to the most easily oxidized base, which is guanosyl; at the second stage, efficient RET from tryptophan may occur with restoration of the diamagnetic base; and at the next stage, intramolecular electron-transfer takes place with the formation of a neutral tyrosyl radical that possibly might be detected by electron spin resonance (ESR) in histone proteins that are tightly packed around DNA in cells.

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