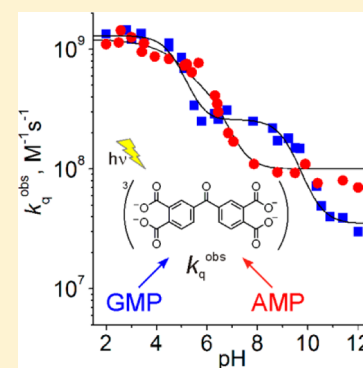


Oxidation of Purine Nucleotides by Triplet 3,3',4,4'-Benzophenone Tetracarboxylic Acid in Aqueous Solution: pH-Dependence

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ABSTRACT: The photo-oxidation of purine nucleotides adenosine-5'-monophosphate (AMP) and guanosine-5'-monophosphate (GMP) by 3,3',4,4'-benzophenone tetracarboxylic acid (TCBP) has been investigated in aqueous solutions using nanosecond laser flash photolysis (LFP) and time-resolved chemically induced dynamic nuclear polarization (CIDNP). The pH dependences of quenching rate constants and of geminate polarization are measured within a wide range of pH values. As a result, the chemical reactivity of reacting species in different protonation states is determined. In acidic solution (pH < 4.9), the quenching rate constant is close to the diffusion-controlled limit: $k_q = 1.3 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (GMP), and $k_q = 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (AMP), whereas in neutral and basic solutions it is significantly lower: $k_q = 2.6 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (GMP, 4.9 < pH < 9.4), $k_q = 3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ (GMP, pH > 9.4), $k_q = 1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (AMP, pH > 6.5). Surprisingly, the strong influence of the protonation state of the phosphoric group on the oxidation of adenosine-5'-monophosphate is revealed: the deprotonation of the AMP phosphoric group (6.5) decreases the quenching rate constant from $5.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (4.9 < pH < 6.5) to $1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ (pH > 6.5).



INTRODUCTION

Free radicals, resulting from electron transfer, are often observed in living biological systems to give rise to many important, functional processes. Nucleic acids and their building blocks are involved in the pathological damage of DNA due to electron removal from DNA bases.^{1–3} This makes the characterization of the radicals and the study of their reactions with nucleotides extremely important. Unfortunately, optical techniques often suffer from insufficient spectral resolution and do not allow unambiguous identification of the radical intermediates of purine nucleotides,^{4–6} while their direct EPR detection under physiological conditions is usually problematical because of very short lifetimes of such radicals. Most of the observations of the radicals derived from purine bases are performed by EPR techniques at low temperatures.^{7–10} We found only limited examples of the detection of radicals derived from purines in solution by means of EPR.^{11,12} In order to study the chemical reactivity of two purine nucleotides, adenosine-5'-monophosphate (AMP) and guanosine-5'-monophosphate (GMP), toward 3,3',4,4'-benzophenone tetracarboxylic acid (TCBP) in the triplet-excited state in aqueous solutions of different pH at room temperature and to provide further information on the acid–base properties of radical intermediates observed in the photo-oxidation reactions of purines in aqueous solution, we have combined two techniques, namely the time-resolved laser flash photolysis (LFP) and the time-resolved chemically induced dynamic nuclear polarization (CIDNP). The CIDNP is the name

given to the nonequilibrium nuclear spin state populations produced in chemical reactions that involve radical pair intermediates. The CIDNP, detected as enhanced absorptive or emissive signals in the NMR spectra of reaction products, has been exploited for more than the last 40 years to characterize transient free radicals and their reaction mechanisms. This paper reports the results of studying the photoinduced oxidation of GMP and AMP with the expectation that the information obtained will provide a basis for understanding reactions associated with electron removal from compounds of this important class.

EXPERIMENTAL SECTION

Chemicals. Guanosine-5'-monophosphate, adenosine-5'-monophosphate, and adenosine were used as received from Fluka. 3,3',4,4'-Benzophenone tetracarboxylic acid, DCl, NaOD (30% solution in D₂O), and D₂O were purchased from Sigma-Aldrich and were used as received.

Laser Flash Photolysis. The samples, placed in a rectangular quartz cuvette (1 × 1 cm²), were irradiated by the pulses of a Lambda Physik LPX-120 XeCl excimer laser (308 nm, pulse energy up to 100 mJ). Monitoring was performed using a 150 W xenon lamp, a Hamamatsu R928 photomultiplier tube, an OBB/PTI

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Table 1. List of Compounds

Compound	Abbreviation	Structure	pK_a	pK_a^*
3,3',4,4'-benzophenone tetracarboxylic acid	TCBP		$pK_{a1}=2.9$; $pK_{a2}=4.9$ ¹³	$pK_{a1}^*=2.7$; $pK_{a2}^*=4.8$
Guanosine 5'-monophosphate	GMP		$pK_{a1}=2.4$; $pK_{a2}=6.5$ ^(a) ; $pK_{a3}=9.4$ ¹⁴	$pK_{a1}^*=2.1$; $pK_{a2}^*=6.5$; $pK_{a3}^*=9.7$
Adenosine 5'-monophosphate	AMP		$pK_{a1}=4.0$; ¹⁴ $pK_{a2}=6.5$ ^(a)	$pK_{a1}^*=3.9$; $pK_{a2}^*=6.5$
Adenosine	Ado		$pK_{a1}=3.5$ ¹⁵	$pK_{a1}^*=3.3$

^a pK_a values of AMP and GMP phosphoric groups were determined by chemical shift titration.

monochromator model 101/102, and a digital storage oscilloscope 9410A LeCroy interfaced to a PC.

In LFP measurements, the concentration of TCBP was 1×10^{-4} M, allowing us to avoid triplet-triplet annihilation. All the samples were prepared in buffered aqueous solutions at room temperature and purged with argon for 15 min prior to irradiation. The buffer solutions with a concentration of 0.01 M were prepared in doubly distilled H₂O with (a) HCl-KH₂PO₄, pH = 3.0–5.0; (b) KH₂PO₄-Na₂HPO₄, pH = 5.0–9.0; (c) Na₂HPO₄-NaOH, pH = 9.0–11.0. pH < 3 and pH > 11 were adjusted with HCl and NaOH, respectively.

Time-Resolved CIDNP. Our setup for TR-CIDNP measurements has already been described in detail.¹⁶ The samples purged with pure nitrogen gas and sealed in a standard NMR Pyrex ampule were irradiated by the pulses of a COMPEX Lambda Physik XeCl excimer laser (wavelength 308 nm, output pulse energy up to 150 mJ) in the probe of a 200 MHz Bruker DPX-200 NMR spectrometer (magnetic field 4.7 T, resonance frequency of protons 200 MHz). Light to the sample was guided using the optical system containing a spherical lens, prism, and light-guide glass fiber (diameter 5 mm). The TR-CIDNP spectra were obtained in the following way: the saturation pulses of the broadband homonuclear decoupler—the laser pulse triggered by the spectrometer—a detecting radio frequency (RF) pulse of 1 μ s duration. The laser pulse was synchronized with the front edge of the RF pulse. As the background signals from Boltzmann polarization were suppressed by saturation pulses, in the CIDNP spectra, only the NMR signals from the polarized products of the cyclic photochemical reaction appear.

All the ¹H NMR measurements were performed in D₂O. The acidity of NMR samples was varied using small amounts of DCl or NaOD. As, in this case, the H₂O calibrated pH-meter was used to measure pH in D₂O solutions, the pH readings correspond to

the so-called pH* values. Thus, the NMR and TR-CIDNP data were obtained from pK_a^* values rather than from normal pK_a according to the formula $pK_a = 0.929pK_a^* + 0.42$.¹⁷

The concentration of TCBP used in CIDNP experiments was 2×10^{-3} M. It was chosen to avoid sample depletion under laser irradiation. The concentrations of quenchers amounted to 10.0×10^{-3} M (GMP, Ado), and to 20.0×10^{-3} M (AMP), respectively.

The structures and abbreviations of the compounds used in this work are listed in Table 1.

RESULTS AND DISCUSSION

The abbreviations GH⁺, G, and G(-H)⁻ denote the cationic, neutral, and anionic forms of the GMP guanyl moiety, AH⁺ and A denote the cationic and neutral forms of the adenyly moiety of either AMP or Ado, and (HPO₄⁻) and (PO₄²⁻) are used to discriminate between the two protonation states of the nucleotide phosphoric group. The pH dependent protonation states of reactive species together with the acidity constants employed in calculations are listed in Tables 2 and 1, correspondingly. The equilibrium concentrations of the protonation forms of TCBP were calculated using two mean ground-state acidity constants $pK_{a1} = 2.9$ and $pK_{a2} = 4.9$ for H₂O,¹³ and $pK_{a1}^* = 2.7$ and $pK_{a2}^* = 4.8$ for D₂O.

Laser Flash Photolysis. The absorption of GMP and AMP at 308 nm with the concentrations chosen is much smaller than that of TCBP. Thus, after the irradiation of a solution containing TCBP, and GMP or AMP, TCBP triplets are generated first, and then quenching occurs. Wavelengths of 590 nm (pH < $pK_{a2}^{\text{TCBP}} = 4.9$) and 550 nm (pH > $pK_{a2}^{\text{TCBP}} = 4.9$)¹⁸ were taken to measure the decay of triplet TCBP. Under these conditions, the triplet-excited dye exhibits absorption maxima. Since the concentration of TCBP is low (1×10^{-4} M) and the triplet-triplet annihilation is negligible, the triplet-excited TCBP decay in the absence of a quencher follows

Table 2. Rate Constants (k_{qi}) of TCBP Triplet Quenching by Guanosine 5'-Monophosphate (GMP), Adenosine 5'-Monophosphate (AMP), and Adenosine (Ado)

quencher	pH region	reactant pair	$k_{qi}, M^{-1} s^{-1}$
GMP	pH < 4.9	TCBPH ₄ or TCBPH ₂ ²⁻ , and GH ⁺ or G ^a	1.3×10^9
	4.9 < pH < 9.4	TCBP ⁴⁻ and G	2.6×10^8
	pH > 9.4	TCBP ⁴⁻ and G(-H) ⁻	3.5×10^7
AMP	pH < 2.9	TCBPH ₄ and AH ⁺ (HPO ₄ ⁻) ^b	1.2×10^9
	2.9 < pH < 4.0	TCBPH ₂ ²⁻ and AH ⁺ (HPO ₄ ⁻)	1.1×10^9
	4.0 < pH < 4.9	TCBPH ₂ ²⁻ and A(HPO ₄ ⁻) ^b	1.0×10^9
	4.9 < pH < 6.5	TCBP ⁴⁻ and A(HPO ₄ ⁻)	5.0×10^8
	pH > 6.5	TCBP ⁴⁻ and A(PO ₄ ²⁻) ^b	1.0×10^8
Ado ^c	pH < 2.9	TCBPH ₄ and AH ⁺	4.9×10^9
	2.9 < pH < 3.5	TCBPH ₂ ²⁻ and AH ⁺	6.8×10^9
	3.5 < pH < 4.9	TCBPH ₂ ²⁻ and A	4.0×10^9
	pH > 4.9	TCBP ⁴⁻ and A	6.0×10^8

^aThe change of quenching rate constant caused by deprotonation of either GH⁺ or TCBPH₄ does not exceed experimental error. ^bThe abbreviation AH⁺(HPO₄⁻) means that the AMP adenyly moiety is charged positively and the AMP phosphoric group is charged negatively; in A(HPO₄⁻) the adenyly moiety is neutral and the phosphoric group is charged negatively; A(PO₄²⁻) denotes that the adenyly moiety is neutral and the phosphoric group is doubly negatively charged, i.e., fully deprotonated. ^cData taken from ref 18.

the first-order kinetics with $k_d = (5.5-8) \times 10^5 s^{-1}$.^{18,19} In the presence of a quencher, the reaction between the triplet TCBP and the quencher occurs with the pseudo-first-order rate constant k_{obs} proportional to the concentration of the quencher, [Q]; $k = k_q^{obs}[Q]$, where k_q^{obs} is a quenching rate constant. Transient absorption decays of ³TCBP with an increase in concentration of GMP and the corresponding Stern–Volmer relation are shown in Figure 1a. The pH dependences of the quenching rate constant k_q^{obs} for GMP and AMP are presented in Figure 1b.

As seen from Figure 1b, the observed pH dependences can be divided into several regions with boundaries at the corresponding pK_a values of the starting compounds that determine the nature of the reacting species.²⁰ In the pH region selected, each pair of reagents, TCBP in the triplet-excited state and the quencher, is characterized by a quenching rate constant (k_{qi}). The observed quenching rate constant k_q^{obs} is calculated as the sum of k_{qi} multiplied by the molar fractions of the dye and the quencher. The molar fractions of the reagents are determined by the pH value of the aqueous solution and by the pK_a values of reactants.

The first protonation state of the GMP guanyl moiety is characterized by the acidity constant pK_{a1} = 2.4. The measurements were carried out at pH > 2, and the observed quenching rate constant was pH-independent up to pH ~ 3.5 within experimental accuracy. So, we were unable to discriminate between all possible reactant pairs in the acidic region (TCBPH₄ and GH⁺, TCBPH₄ and G, TCBPH₂²⁻ and GH⁺, TCBPH₂²⁻ and G). Surprisingly, for AMP, we have failed to simulate the pH dependence of the observed quenching rate constant taking into account the pK_a values of the adenyly moiety only; account should be taken of the second pK_a value of the AMP phosphoric group (pK_a = 6.5). In the case of GMP, there was no need to consider the protonation state of the phosphoric group in our simulations of LFP and CIDNP data. Thus, the pH dependences of the observed quenching rate constant (k_q^{obs}) were simulated according to eqs 1 (for GMP) and 2 (for AMP). The k_{qi} values obtained by the best fits (solid lines, Figure 1b) are shown in Table 2 together with the corresponding pairs of reactants.

$$\begin{aligned}
 k_q^{obs} = & k_{q1} \times \frac{[H^+]^2}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{[H^+]^2}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}} \\
 & + k_{q1} \times \frac{[H^+]^2}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{K_{a1}^{GMP}[H^+]}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}} \\
 & + k_{q1} \times \frac{K_{a1}^{TCBP}[H^+]}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{[H^+]^2}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}} \\
 & + k_{q1} \times \frac{K_{a1}^{TCBP}[H^+]}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{K_{a1}^{GMP}[H^+]}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}} \\
 & + k_{q2} \times \frac{K_{a1}^{TCBP}K_{a2}^{TCBP}}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{K_{a1}^{GMP}[H^+]}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}} \\
 & + k_{q3} \times \frac{K_{a1}^{TCBP}K_{a2}^{TCBP}}{[H^+]^2 + K_{a1}^{TCBP}[H^+] + K_{a1}^{TCBP}K_{a2}^{TCBP}} \\
 & \times \frac{K_{a1}^{GMP}K_{a3}^{GMP}}{[H^+]^2 + K_{a1}^{GMP}[H^+] + K_{a1}^{GMP}K_{a3}^{GMP}}
 \end{aligned} \quad (1)$$

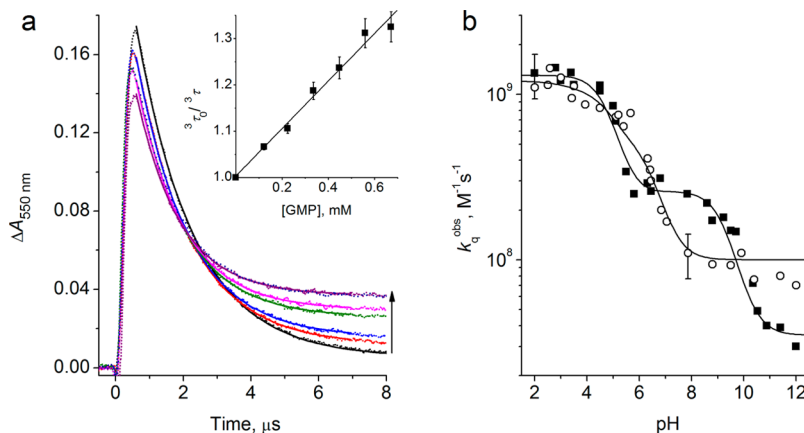


Figure 1. (a) Transient absorption decays ($\lambda_{obs} = 550$ nm) of triplet TCBP (1×10^{-4} M) in the presence of GMP (concentration increasing from bottom to top: 0 to 6.7×10^{-4} M) in water at pH = 6.8. Inset: Stern–Volmer plot. (b) pH dependences of the observed quenching rate constant for the reaction of triplet TCBP with GMP (squares) and AMP (circles). Solid lines are the simulations from eqs 1 and 2.

$$\begin{aligned}
 k_q^{\text{obs}} = & k_{q1} \times \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{a1}^{\text{TCBP}}[\text{H}^+] + K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}} \\
 & \times \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{a1}^{\text{AMP}}[\text{H}^+] + K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}} \\
 & + k_{q2} \times \frac{K_{a1}^{\text{TCBP}}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}^{\text{TCBP}}[\text{H}^+] + K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}} \\
 & \times \frac{[\text{H}^+]^2}{[\text{H}^+]^2 + K_{a1}^{\text{AMP}}[\text{H}^+] + K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}} \\
 & + k_{q3} \times \frac{K_{a1}^{\text{TCBP}}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}^{\text{TCBP}}[\text{H}^+] + K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}} \\
 & \times \frac{K_{a1}^{\text{AMP}}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}^{\text{AMP}}[\text{H}^+] + K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}} \\
 & + k_{q4} \times \frac{K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}}{[\text{H}^+]^2 + K_{a1}^{\text{TCBP}}[\text{H}^+] + K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}} \\
 & \times \frac{K_{a1}^{\text{AMP}}[\text{H}^+]}{[\text{H}^+]^2 + K_{a1}^{\text{AMP}}[\text{H}^+] + K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}} \\
 & + k_{q5} \times \frac{K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}}{[\text{H}^+]^2 + K_{a1}^{\text{TCBP}}[\text{H}^+] + K_{a1}^{\text{TCBP}}K_{a2}^{\text{TCBP}}} \\
 & \times \frac{K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}}{[\text{H}^+]^2 + K_{a1}^{\text{AMP}}[\text{H}^+] + K_{a1}^{\text{AMP}}K_{a2}^{\text{AMP}}} \quad (2)
 \end{aligned}$$

In acidic solutions, GMP and AMP quench the triplet-excited TCBP with $k_{q1} = 1.3 \times 10^9$ and $k_{q1} = 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, respectively, which is close to the diffusion-controlled limit. Deprotonating TCBPH_4 ($\text{p}K_a = 2.8$) to form TCBPH_2^{2-} has no significant effect on the quenching rate. The first steep decrease of k_q^{obs} in the reaction of TCBP triplets with GMP is due to the deprotonation of TCBPH_2^{2-} into TCBP^{4-} ($\text{p}K_a = 4.9$). The second change is characterized by the deprotonation of a guanyl base ($\text{p}K_a = 9.4$). It is impossible to determine the quenching mechanism from our LFP data.

The deprotonation of TCBPH_4 and TCBPH_2^{2-} has no noticeable effect on the reaction rate of triplet-excited TCBP quenching by AMP. In contrast to the case of GMP, the deprotonation of the phosphoric group ($\text{p}K_a = 6.5$) decreases the quenching reaction rate constant by a factor of 5. Compared to the photoreaction between the triplet-excited TCBP and Ado studied in ref 18, one can see that the presence of the negatively charged phosphoric group reduces the quenching rate constant k_q^{obs} 4–6 times over the entire pH range.

Although adenosine is generally considered less reactive than guanosine (as guanosine has a lower reduction potential²¹), our

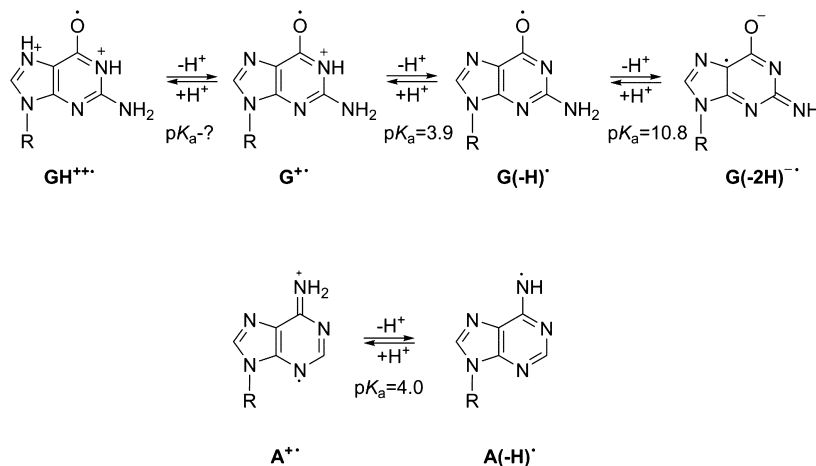
results indicate that AMP is readily oxidized by $^3\text{TCBP}$ within the pH range from 2 to 12. Moreover, AMP is about three times more reactive than GMP at $4.0 < \text{pH} < 4.9$ and at $\text{pH} > 9.4$.

CIDNP Measurements. Since the EPR technique is not usually applicable for low concentrations and for short lifetimes of the radicals under study in aqueous solution at room temperature, the adenosyl and guanosyl radicals could be detected in solutions by transient optical spectroscopy.^{4,6} However, using optical detection it is difficult to distinguish the cationic, neutral, or anionic forms of nucleotide radicals due to the overlapping of the transient optical spectra of the different protonation forms of these radicals. Therefore, we used the TR-CIDNP technique to characterize radical intermediates resulting from the quenching of the triplet-excited TCBP by purine nucleotides adenosine-5'-monophosphate and guanosine-5'-monophosphate and also by nucleoside adenosine (Ado) to confirm the influence of the protonation state of the phosphoric group on the quenching rate constant. The characterization of radical intermediates is of essence with respect to the quenching mechanism, which is unavailable from the LFP data. The quenching of the triplet-excited TCBP by GMP or AMP (Ado) results in the formation of a spin-correlated radical pair in the triplet state. The triplet-singlet conversion of the radical pair caused by magnetic interactions in the pair enables back-electron or hydrogen transfer, leading to the restoration of polarized initial compounds. The TR-CIDNP experimental facility can be used to detect the so-called geminate CIDNP arising at the nanosecond time scale in geminate products. The magnetic resonance parameters of the geminate radical pair are encoded in both the amplitude and the phase of geminate CIDNP signals.

The structure of the adenosyl and guanosyl radicals formed in the quenching reaction strongly depends on the pH of the aqueous solution (Chart 1). In the present paper, the symbols G^{*+} and A^{*+} , respectively, denote the cations of guanosyl and adenosyl radicals, $\text{G}(-\text{H})^\bullet$ and $\text{A}(-\text{H})^\bullet$ for the neutral forms of these radicals, and $\text{G}(-2\text{H})^{\bullet-}$ for guanosine radical anion. These notations refer only to the protonation state of the purine base and do not include the protonation state of the phosphoric group.

The CIDNP spectra detected in the photoreaction between TCBP and the quencher (GMP or AMP) immediately after the laser pulse at different pH values are shown in Figure 2. The amplitude and the sign of geminate polarization are observed to change with varying pH. The net CIDNP sign, Γ , is determined in terms of simple CIDNP rules,²³ i.e., $\Gamma = \text{sgn}(\Delta g) \times \text{sgn}(A)$ for

Chart 1. Structures of Adenosyl and Guanosyl Radicals ($\text{p}K_a$ Values Are Taken from Refs 14 and 22)



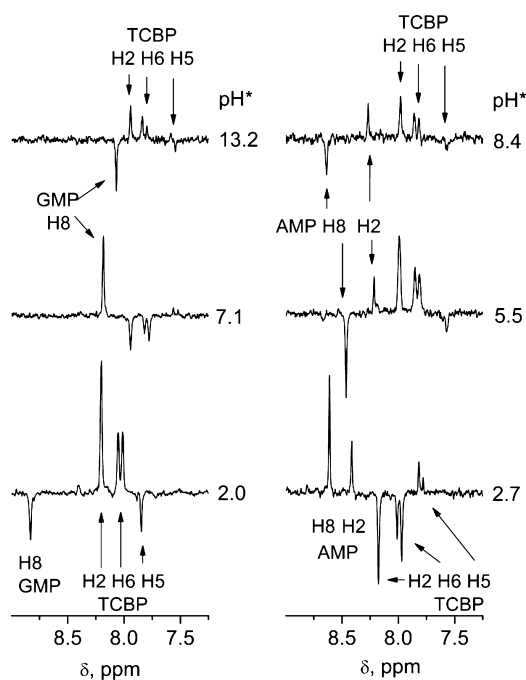


Figure 2. 200 MHz ^1H CIDNP spectra obtained by photoreaction between 2 mM TCBP and (left) 10.0 mM GMP; (right) 20.0 mM AMP in D_2O . All spectra were recorded immediately after the laser pulse with a detecting RF-pulse of 1 μs . The relative intensities of the spectra shown in each column correspond to those observed experimentally. The scaling of left- and right-column spectra are independent.

the geminate recombination product and for the radical pair triplet precursor. In this case, $\Delta g = g_1 - g_2$ is the difference in the radical g -factors of the radical pair, and A is a hyperfine coupling constant (HFCC) for the nucleus concerned.

Figures 3–5 show the behavior of both the geminate polarization TCBP and the quenchers GMP (Figure 3), AMP

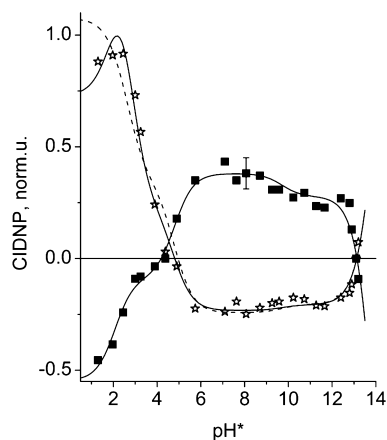


Figure 3. pH^* dependences of geminate CIDNP intensity in the photoreaction of triplet TCBP with GMP for GMP H8 (solid squares) proton and for TCBP H6 (stars) proton. Solid lines: simulations from eq 4.

(Figure 4), and Ado (Figure 5). The vertical scaling factors were chosen so that the maximum of the calculated CIDNP value for the TCBP H6 proton in each calculated curve was equal to unity.

In the GMP CIDNP spectra only the H8 proton is polarized. No polarization was observed for ribose protons. The polarization of the GMP H8 proton was negative (emission) under

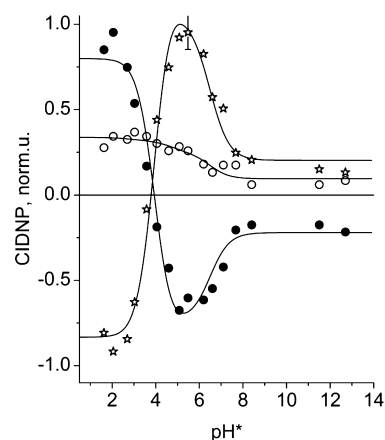


Figure 4. pH^* dependences of geminate CIDNP intensity in the photoreaction of triplet TCBP with AMP for AMP H8 (solid circles) and H2 (open circles) protons and for TCBP H6 (stars) proton. Solid lines: simulations from eq 4.

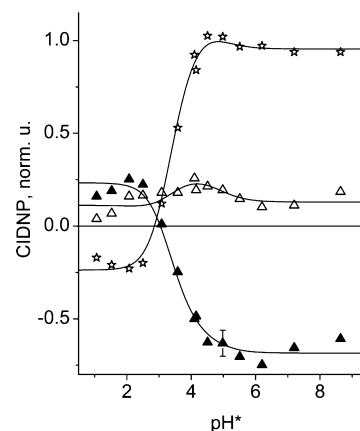


Figure 5. pH^* dependences of geminate CIDNP intensity in the photoreaction of triplet TCBP with Ado for Ado H8 (solid triangles) and H2 (open triangles) protons and for TCBP H6 (stars) proton. Solid lines: simulations from eq 4.

acidic conditions and in the extremely basic solution ($\text{pH}^* > 13$), and positive (enhanced absorption) in neutral and basic solutions. A consistent polarization sign inversion is observed for TCBP signals. The HFCC sign reported for the H8 proton of the cationic, neutral and anionic guanosyl radicals is negative.^{10,20} In aqueous glass, at 77 K the g -factors of the guanosyl radicals were measured to be 2.0037 (guanosyl radical cation), 2.0034 (guanosyl neutral radical), and 2.0036 (guanosyl anion radical).¹⁰ The g -factor of the TCBP radical anion is 2.0035.²⁴ It is concluded then that the polarization sign inversion with varying pH is due to the change of the sign of g -factor difference in the dye and guanosyl radical pairs. The polarization signs correspond to the guanosyl radical cation $\text{G}^{\bullet+}$ at $\text{pH}^* < 4.8$, the guanosyl neutral radical $\text{G}(-\text{H})^{\bullet}$ at $4.9 < \text{pH}^* < 13$, and the guanosyl radical anion $\text{G}(-2\text{H})^{\bullet-}$ in extremely basic solutions ($\text{pH}^* > 13$) (Chart 1). In our previous work, 2,2'-dipyridyl was used as a dye ($g = 2.0030$ for the neutral radical dipyridyl²⁵). The deprotonation of the guanosyl radical cation caused no changes in the sign of the g -factors difference and thus no inversion of the CIDNP sign.²⁰ One more guanosyl radical mentioned in the literature is the dication radical $\text{GH}^{\bullet++}$. The data on the g -factor and HFCCs of this radical are unavailable. In our measurements, we were able to differentiate radical cations and dications because

they were involved in a degenerate electron exchange reaction with a parent molecule (guanosine cation for radical dication, and neutral guanosine for radical cation). This reaction proceeds with different efficiency for the two pairs of participants, $\text{GH}^{\bullet\bullet\bullet}/\text{GH}^{\bullet}$ and $\text{G}^{\bullet\bullet}/\text{G}$, which is reflected in CIDNP kinetics. In the present study, simulating the CIDNP pH, we have taken into account the GMP radical dication and cation (vide supra) with a corresponding $\text{p}K_{\text{a}}^* = 2.1$; otherwise, it would be impossible to obtain good agreement between experiment and the simulation of CIDNP the pH dependence for TCBP (dashed line in Figure 3).

For adenosine, the two radicals are known, i.e., the neutral one and the radical cation (Chart 1). The *g*-factor for the adenosyl radical cation in aqueous glass at 77 K is 2.0034, and that for the adenosyl neutral radical is 2.0037.⁹ The radical cation $\text{A}^{\bullet\bullet}$ has negative HFCCs for the H8 and H2 protons; the neutral radical $\text{A}(-\text{H})^{\bullet}$ has negative HFCC for the H8 proton and the positive one for the H2 proton.^{9,22} The polarization of the AMP H8 proton was positive (enhanced absorption) under acidic conditions and negative (emission) in neutral and basic solutions. A corresponding sign change is also observed for TCBP protons. The polarization sign of the AMP H2 proton remains positive over the entire pH range. These observations are consistent with CIDNP originating from the reactions between the dye radical and the radical cation $\text{A}^{\bullet\bullet}$ at $\text{pH}^* < 4.9$, and between the dye radical and the neutral radical $\text{A}(-\text{H})^{\bullet}$. The change of the Δg sign caused by substitution of the adenosyl radical cation by the neutral radical results in the inversion of the polarization sign of both the AMP H8 and the TCBP protons, and keeps the polarization sign for the AMP H2 proton for which the HFCC and Δg signs change simultaneously.

The change in the polarization sign of GMP and AMP in the presence of N3-carboxymethyl lumiflavin has been first revealed by Stob et al. with the help of the cw-CIDNP technique.^{22,26} The mechanism involves the so-called “pair substitutions effect”, namely the deprotonation of adenosyl radical cation to the neutral radical for AMP and the deprotonation of guanosyl dication radical cation radical for GMP. From these data, the $\text{p}K_{\text{a}}$ value of the AMP radical cation was estimated to be 4.

The approach used to simulate the pH dependence of $k_{\text{q}}^{\text{obs}}$ was applied to the pH dependences of geminate CIDNP intensities. In general, the amplitude of the geminate CIDNP depends on

two factors: the concentration of radicals (in other words, how effective is the dye triplets quenching) and magnetic interactions in the radical pair. The quenching efficiency (q_i) depends on the quencher concentration in a certain protonated state (c_{q}), on the intrinsic lifetime of the dye triplet (τ), and on the quenching rate constants (k_{qi}) for a pair of reactants, and it is described by the Stern–Volmer equation:

$$q_i = \frac{1}{\frac{1}{k_{\text{qi}}\tau c_{\text{q}}} + 1} \quad (3)$$

In addition to the quenching rate constant (k_{qi}), each pair of reagents is characterized by polarization generated by this pair (p_i being the NMR enhancement factor). Thus, the total CIDNP intensity (I) can be simulated as the sum of partial intensities $p_i \times q_i$ multiplied by the molar fractions (α) of the quencher and the dye:

$$I = \sum_i p_i \times q_i \times \alpha(\text{quencher}) \times \alpha(\text{dye}) \quad (4)$$

The products $p_i \times q_i$ are fitting parameters, whose values determine the intensity of the geminate CIDNP signals depending on pH. The best-fit values of these products are shown in Table 3. The inflection points of CIDNP titration curves for the purine derivatives studied are correlated with the $\text{p}K_{\text{a}}$ values of the protonated states of the quencher and the dye and with the pH dependencies of the observed quenching rate constant $k_{\text{q}}^{\text{obs}}$.

The pH dependence of the geminate CIDNP intensity obtained in the photoreaction of triplet TCBP with GMP (Figure 3) has the following main features: the CIDNP minimum (maximum of absolute value) for the GMP H8 proton is observed under acidic solutions; the negative polarization for the GMP H8 increases with decreasing acidity until absorption is observed at $\text{pH}^* > 4$; the deprotonation of the GMP phosphoric group ($\text{p}K_{\text{a}} = 6.5$) has no effect on the titration curve behavior; a decrease in CIDNP intensity in basic solution is related to the deprotonation of the guanosyl moiety ($\text{p}K_{\text{a}}^* = 9.7$). The decrease in CIDNP intensity for both GMP and TCBP at $\text{pH}^* > 13$ and the consequent change of CIDNP sign at $\text{pH}^* = 13.2$ are attributed to the formation of the guanosyl radical anion.

In the case of AMP, the CIDNP maximum is also observed in acidic solutions (Figure 4). The AMP H8 positive polarization

Table 3. Relative CIDNP Magnitudes ($p_i \times q_i$) for Different Reactant Pairs

quencher	pH* region	reactant pair	nucleotide radical	$p_i \times q_i$		
				H8	H2	H6 (TCBP)
GMP	$\text{pH}^* < 2.1$	TCBPH ₄ and GH ⁺	GH ^{•••}	−0.54	n/a	0.74
	$2.1 < \text{pH}^* < 2.7$	TCBPH ₄ and G	G ^{••}	−0.23		1.67
	$2.7 < \text{pH}^* < 4.8$	TCBPH ₂ ^{2−} and G		−0.08		0.24
	$4.8 < \text{pH}^* < 9.7$	TCBP ^{4−} and G	G(−H) [•]	0.38		−0.23
	$9.7 < \text{pH}^* < 13^{\text{a}}$	TCBP ^{4−} and G(−H) [−]		0.28		−0.19
	$\text{pH}^* > 13$	TCBP ^{4−} and G(−H) ^{•−}	G(−2H) ^{•−}	−2.00		1.41
AMP	$\text{pH}^* < 3.9$	TCBPH ₄ or TCBPH ₂ ^{2−} and AH ⁺ (HPO ₄ [−])	A ^{••} (HPO ₄ [−])	0.80	0.31	−0.83
	$3.9 < \text{pH}^* < 4.8$	TCBPH ₂ ^{2−} and A(HPO ₄ [−])	A(−H) [•] (HPO ₄ [−])	−0.90	0.32	1.37
	$4.8 < \text{pH}^* < 6.5$	TCBP ^{4−} and A(HPO ₄ [−])		−0.73	0.23	1.02
	$\text{pH}^* > 6.5$	TCBP ^{4−} and A(PO ₄ ^{2−})	A(−H) [•] (PO ₄ ^{2−})	−0.22	0.08	0.20
Ado	$\text{pH}^* < 3.3$	TCBPH ₄ or TCBPH ₂ ^{2−} and AH ⁺	A ^{••}	0.24	0.11	−0.23
	$3.3 < \text{pH}^* < 4.8$	TCBPH ₂ ^{2−} and A	A(−H) [•]	−1.11	0.28	0.61
	$\text{pH}^* > 4.8$	TCBP ^{4−} and A		−0.95	0.13	0.68

^aAt pH > 13 guanosyl radical anion, G(−2H)^{•−} is formed due to the interaction between the neutral radical G(−H)[•] and OH[−], which accelerates the deprotonation of the neutral radical.

decreases with decreasing acidity until emission is observed at $\text{pH}^* > 4$. A maximum of emissive signal is reached at $\text{pH}^* = 5.5$. Above this pH, the CIDNP signal of H8 decreases with an inflection point equal to $\text{p}K_a$ of the phosphoric group (6.5), which corresponds to the lower quenching rate constant in the case of the fully deprotonated phosphoric group. The CIDNP effect of H2 undergoes almost no changes up to $\text{pH}^* 5.5$ and decreases with deprotonation of $-\text{HPO}_4^-$. The influence of the protonation state of the phosphoric group is confirmed by the absence of any change in CIDNP intensity obtained in the photoreaction of the triplet TCBP with Ado (Figure 5) at $\text{pH}^* > 5.9$.

Consider now the reaction mechanism following from CIDNP data. The sign of nuclear polarization originating from the photoreaction of adenosine cation $\text{AH}^+(\text{HPO}_4^-)$ at $\text{pH}^* < 3.9$ corresponds to CIDNP formed in the radical pair of dye radical and adenosyl radical cation $\text{A}^+(\text{HPO}_4^-)$. This radical could be produced via hydrogen transfer from AMP, proton coupled electron transfer (PCET), or electron transfer with consequent deprotonation of the adenosyl radical dication. The hydrogen transfer is ruled out because of an essentially high quenching rate constant of $1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, untypical of this type of reaction. The adenosyl radical dication, which is a possible product of electron transfer from adenosine cation, has no mention in the literature, and, therefore, has, probably, a very low $\text{p}K_a$ value, leading to its fast deprotonation into the adenosyl radical cation, which gives rise to the CIDNP effects observed. The adenosyl radical cation could also be formed via PCET reaction. Thus, we cannot discriminate between electron transfer and PCET as a possible mechanism of triplet-excited TCBP quenching by adenosine cation.

In the case of neutral adenosine as a quencher ($\text{pH} > 4$), the electron transfer leads to the formation of an adenosyl radical cation with $\text{p}K_a = 4.0$. The radicals with similar $\text{p}K_a$ are known to have a lifetime of about $1 \mu\text{s}$,^{27,28} which is long enough for the geminate CIDNP to be formed in the primary radical before its deprotonation into conjugated base. In the case of the electron transfer, the CIDNP observed in our experiments should correspond to that formed with the participation of radical cation and without any change in CIDNP sign. Thus, the electron transfer is ruled out as the mechanism of triplet-excited TCBP quenching by neutral adenosine. The probable mechanisms are either the hydrogen transfer or PCET leading to the formation of a neutral adenosyl radical. The role of the protonation state of the phosphoric group in the quenching reaction under study, though confirmed by CIDNP measurements, still remains unclear.

For GMP, it was necessary to take into account the formation of dication and cation radicals in acidic solution ($\text{pH}^* < 4.8$). These radicals are the products of electron transfer from guanosine cation and neutral guanosine, respectively, and therefore, this mechanism follows from the reactions between TCBPH_4 and GH^+ , TCBPH_4 and G, and TCBPH_2^{2-} and G. The reaction of the fully deprotonated triplet-excited TCBP^{4-} with the neutral guanosine results in the formation of neutral guanosyl radical, which is manifested in the positive polarization of GMP H8, and the corresponding negative polarization of TCBP H2 and H6. The polarization sign does not change upon deprotonation of neutral guanosine into its anion at $\text{pH}^* > 9.7$. The neutral guanosyl radical could be formed only via electron transfer from guanosine anion. Thus, a rate constant of $3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ measured by us for the reaction between TCBP^{4-} and $\text{G}(-\text{H})^-$ corresponds to the quenching via electron transfer, and it is the lowest rate constant for the reaction of this mechanism ever obtained by us.^{20,29} The change in CIDNP sign

at $\text{pH} > 13$, consistent with the CIDNP originating from the reactions of guanosyl anion radical, confirms that this radical is derived from the reaction between the guanosyl neutral radical and hydroxyl ions rather than from quenching: only with hydroxyl ions concentration higher than 0.1 M is the deprotonation rate of $\text{G}(-\text{H})^\bullet$ ($\text{p}K_a = 10.8$) high enough to provide the formation of the $\text{G}(-2\text{H})^\bullet$ radical within the lifetime of the triplet spin-correlated radical pair.

Thus, both the neutral and the anionic guanosines quench the triplet-excited dye to form the neutral guanosyl radical. It is worth noting that a certain inconsistency was found in the obtained data. Namely, despite a 7-fold difference in the reactivities of guanosine in neutral and anionic forms toward the triplet-excited TCBP^{4-} (the quenching rate constants are 2.6×10^8 and $3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$), there is no corresponding difference in the CIDNP intensities detected: $p_i \times q_i$ for the reactant pair TCBP^{4-} and $\text{G}(-\text{H})^-$ is only slightly lower than that for TCBP^{4-} and $\text{G}(-\text{H})^\bullet$, whereas a several times decrease is expected. This decrease of $p_i \times q_i$ should be similar to that observed for AMP when the rate constant k_{qi} was slowed down from $5.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ to $1.0 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$. To clarify this apparent inconsistency, we have measured the dependence of CIDNP intensity on GMP concentration at $\text{pH}^* 7.2$ and 11.7. The CIDNP intensity of the TCBP H6 was chosen for analysis (Figure 6), since at $\text{pH}^* > 9.7$ GMP in the anionic form is

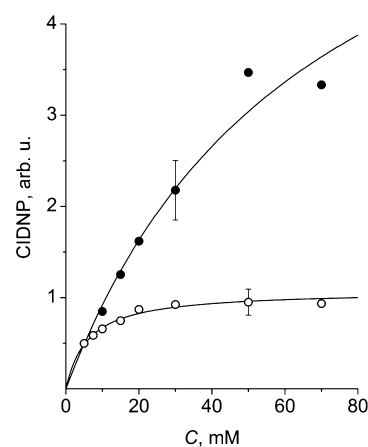


Figure 6. Dependences of geminate CIDNP of the TCBP H6 protons, obtained during photoreaction of GMP with TCBP, on GMP concentration at $\text{pH}^* 7.2$ (open circles) and 11.7 (solid circles). Solid lines: simulations from eq 5.

involved in the reaction of degenerate electron exchange with a neutral GMP radical, and this leads to a decrease in the CIDNP intensity of the GMP H8 proton detected after the laser pulse.

The dependence of CIDNP intensity on the GMP concentration C obeys the equation

$$I = \frac{I_\infty C}{k_q^{-1} \tau^{-1} + C} \quad (5)$$

where k_q is the quenching rate constant, τ is the intrinsic lifetime of dye triplets, and I_∞ is the CIDNP intensity under saturation conditions with $C \gg k_q^{-1} \tau^{-1}$. The dependences in Figure 6 reflect the differences in CIDNP intensities (scaling keeps the relative intensities of signals in CIDNP spectra), as well as those in k_q values: with higher k_q , saturation is observed at lower C . The values of I_∞ and k_q were obtained from the best-fit simulations using eq 5 and $\tau = 1.4 \mu\text{s}$ for the triplet-excited TCBP.³⁰

The estimated values of k_{qi} are $1.4 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ at $\text{pH}^* 7.2$, and $1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$ at $\text{pH}^* 11.7$, which, taking into account the expected 2-fold decrease in rate constant due to the deuterium isotope effect, is in satisfactory agreement with the LFP data. Taking I_{∞} at $\text{pH}^* 7.2$ as unity, at $\text{pH}^* 11.7$ we obtain $I_{\infty} = 6.8$, although in both of the cases, the CIDNP formed from the radical pairs of dye and neutral GMP radicals should result in identical I_{∞} values. We offer the following explanation for the difference in I_{∞} in terms of the reaction mechanism. PCET is the mechanism of the reaction between fully deprotonated triplet excited TCBP and neutral GMP, in which the electron transfer is followed by the proton transfer within the lifetime of the spin-correlated radical pair initially formed in the triplet state. The electron transfer results in the formation of the guanosyl radical cation. Its magnetic resonance parameters are favorable for gaining a singlet character of radical pairs with the negative nuclear spin projection of the H8 of GMP, and the positive nuclear spin projection of H2 and H6 of TCBP. The consequent proton transfer from the guanosyl radical cation with the formation of the neutral guanosyl radical results in Δg sign inversion. Accordingly, the projections of nuclear spins that provide faster triplet-singlet transitions are changed to the opposite ones. The resulting CIDNP signs correspond to that formed with the participation of the neutral guanosyl radical. However, the CIDNP intensity is attenuated to the degree depending on the lifetime of the guanosyl radical cation as compared to the lifetime of the spin-correlated radical pairs. Thus, a detailed analysis of CIDNP effects including CIDNP pH and concentration dependences allowed us to establish the mechanism of the reaction of the GMP triplet-excited TCBP over a wide pH range.

CONCLUSIONS

The photo-oxidation of the purine nucleotides adenosine-5'-monophosphate and guanosine-5'-monophosphate by 3,3',4,4'-benzophenone tetracarboxylic acid has been investigated within the pH range of 2 to 12 in aqueous solution. The pH dependence of the observed quenching rate constant was measured by time-resolved laser flash photolysis and explained in terms of the pK_{a} values of the reactants. As a result, the quenching rate constants for each pair of reactants were determined by simulating the pH dependence of the quenching rate constant observed. In acidic solutions, the rate constant of the photo-oxidation reaction is maximal: $k_{\text{qi}} = 1.3 \times 10^9$ for GMP and $k_{\text{qi}} = 1.2 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ for AMP, and was observed to decrease with increasing pH. The deprotonation of the AMP phosphoric group was revealed to cause a 5-fold decrease in the quenching reaction rate constant. At the same time, the protonation state of the GMP phosphoric group has no effect on the photoreaction.

A detailed study on the pH^* dependence of the geminate CIDNP intensity indicates that the mechanism of the reaction between the triplet TCBP and GMP or AMP depends on the protonation state of the reacting species. The reaction between ${}^3\text{TCBP}^{\text{H}_4}$ and GH^+ , ${}^3\text{TCBP}^{\text{H}_4}$ and G, ${}^3\text{TCBP}^{\text{H}_2^{2-}}$ and G is the electron transfer. We suggest PCET for the reaction between the fully deprotonated ${}^3\text{TCBP}^{4-}$ and neutral guanosine involving the so-called "pair substitution effect", i.e., the deprotonation of the initially formed guanosyl radical cation within the lifetime of the geminate radical pair. The quenching of dye triplets by guanosine anion proceeds via electron transfer with an extremely low rate constant of $3.5 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}$. At $\text{pH}^* > 13$ the concentration of hydroxyl ions is high enough to catalyze the deprotonation of the neutral guanosyl radical formed in basic

solution. At $\text{pH} < 4$ the quenching of ${}^3\text{TCBP}$ by adenosine cation via either electron transfer or PCET results in the formation of the adenosyl radical cation. Hydrogen atom transfer or PCET lead to the formation of the neutral adenosyl radical at $\text{pH} > 4$.

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Notes

The authors declare no competing financial interest.

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