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Reduction of Guanosyl Radical by Cysteine and Cysteine-Glycine Studied by Time-Resolved CIDNP

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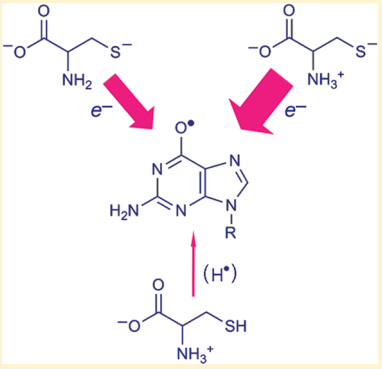
Supporting Information

ABSTRACT: As a model for chemical DNA repair, reduction of guanosyl radicals in the reaction with cysteine or the dipeptide cysteine-glycine has been studied by time-resolved chemically induced dynamic nuclear polarization (CIDNP). Radicals were generated photochemically by pulsed laser irradiation of a solution containing the photosensitizer 2,2′-dipyridyl, guanosine-5′-monophosphate, and the amino acid or peptide. In neutral and basic aqueous solution, the neutral guanosyl radical is formed via electron or hydrogen atom transfer to the triplet excited dye. The rate constants for reduction of guanosyl radical were determined by quantitative analysis of the CIDNP kinetics, which are sensitive to the rates of fast radical reactions. The rate constants vary from $(1.0 \pm 0.3) \times 10^7$ M^{-1} s^{-1} for the thiol form of cysteine to $(1.6 \pm 0.2) \times 10^8$ M^{-1} s^{-1} for the thiolate anion. These values are comparable with corresponding rate constants for reduction of neutral guanosyl radical by tyrosine.



INTRODUCTION

Free-radical-induced damage to DNA is a key issue in radiation chemistry.[1] Interaction of DNA with high-energy radiation results in formation of an electron hole in DNA, which migrates along the DNA chain and ends up on the base with the lowest oxidation potential, guanine.[2] The guanyl cation radical formed in such a way deprotonates under basic or neutral conditions to give rise to the neutral guanyl radical.[3] These two radicals are potential precursors for pathological conditions.[4] The electronic vacancies in the cationic or neutral oxidized guanyl bases may be refilled rather fast via electron transfer from the surrounding protein pool.[5,6] This mechanism, termed "chemical DNA repair",[5,6] efficiently competes with formation of DNA lesions that are targets for enzymatic repair.[5,6] For this chemical DNA repair the amino acids tryptophan, tyrosine, and cysteine were found to be the most efficient reducing agents for guanyl radicals formed in plasmid DNA under γ-irradiation.[5,6]

Thiols have been considered to defend cells against ionizing radiation by two mechanisms called "protection" and "repair".[1,7] The protection mechanism includes scavenging of radicals that could attack a biological target. The repair mechanism consists of restoration of an intact biomolecule from an already formed target radical. For the latter reduction of guanosyl radicals electron transfer or proton-coupled electron transfer has been proposed.[6,8] In particular, the strong attenuation of the yield of radiation-induced breaks in single-stranded DNA in the presence of SH compounds has been reported.[9] It served as indirect evidence for the presence of guanyl radicals, but the mechanism of the repair reaction still remains unclear. Since the concentration of the guanyl radical intermediates formed in the above-mentioned processes is very low, their in situ detection by conventional electron paramagnetic resonance spectroscopy is not feasible at ambient conditions but requires cryogenic temperatures.[10] An alternative approach as applied in the present work is to use indirect detection of the radicals by chemically induced dynamic nuclear polarization (CIDNP) of diamagnetic products of free radical reactions that provides a much higher sensitivity. CIDNP is produced in singlet−triplet transitions of spin-correlated radical pairs and manifests itself as anomalous intensity, emission, or enhanced absorption of NMR spectral lines of the nuclei that have a hyperfine interaction with the unpaired electron in the transient radicals.[11] Strong enhancement factors of the nuclear polarization make the method very sensitive.[12] The time-resolved version of the CIDNP technique (TR-CIDNP) opens the possibility of NMR detection of reaction products formed on a submicrosecond time scale, providing detailed structural information available from high-resolution NMR spectroscopy.[13,14] The possibility to discriminate between different protonation states of the guanosyl radical when they are involved in degenerate electron exchange with the parent diamagnetic molecule makes the method particularly valuable.[14] Using this method, we previously studied the kinetics of reduction of four types of guanosyl radical by the amino acids tyrosine or tryptophan in a wide pH range.[15,16]

The reported oxidation potentials are 1.29 V for guanosine[2] and 0.9 V for cysteine[17] at neutral pH, allowing reduction of guanosyl radical by cysteine. Reduction by cysteine was studied

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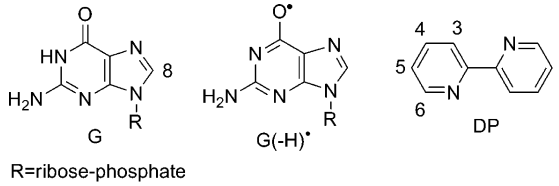
theoretically using density functional theory.[18,19] The predicted mechanism of reduction by cysteine is hydrogen atom transfer (eq 1a) characterized by a low barrier in aqueous medium. Reduction of guanosyl radical via electron transfer from thiolate anion should be even more efficient (eq 1b)

$$G(-H)^{\bullet} + CysSH \xrightarrow{k_r} G + CysS^{\bullet} \quad (1a)$$

$$G(-H)^{\bullet} + CysS^{-} \xrightarrow{k_r} G(-H)^{-} + CysS^{\bullet} \quad (1b)$$

However, direct observation of the reaction of cysteine with target guanosyl radicals (Chart 1) has not been reported. The

Chart 1. Structures of Guanosine-5′-monophosphate (GMP) with Neutral Purine Base G, Neutral Guanosyl Radical G(−H)•, and 2,2′-Dipyridyl DP



aim of the present work is to characterize this reaction using the TR-CIDNP approach. The experimental results obtained here confirm the proposed reaction scheme.

EXPERIMENTAL SECTION

The setup for TR-CIDNP detection has been described previously.[16,20] Samples, sealed in a standard 5 mm Pyrex NMR tube, were irradiated by a COMPEX Lambda Physik XeCl excimer laser in the probe of a 200 MHz Bruker DPX-200 NMR spectrometer. The following pulse sequence was used: radiofrequency saturation pulses−laser pulse−evolution time τ radiofrequency detection pulse−free induction decay. In all kinetic measurements an RF pulse with a duration of 1 μs was used for CIDNP detection. The timing corresponds to the center of the RF pulse (i.e., 0.5 μs for τ = 0) on all CIDNP plots in Figures 2 and 3. The pH of the NMR samples was adjusted by addition of DCl or NaOD. L-Cysteine and cysteine-glycine were purchased from Bachem and used without further purification; 2,2′-dipyridyl (DP), 5′-guanosine monophosphate, DCl, NaOD (30% solution in D_2O), and D_2O (99.9% enriched) were used as received from Sigma-Aldrich. The concentration of DP was 8 mM and of GMP 20 (pH* 7.3), 30 (pH* 8.5), 10 (pH* 9.7), and 5 mM (pH* 11.3). The optical density of the sample at 308 nm was 0.4 (with a 4 mm inner NMR tube diameter as an optical pathway). Since pH measurements were performed in D_2O using a H_2O-calibrated pH meter, the readings correspond to so-called pH* values.[21] For correct calculations of the equilibrium concentrations of the acid and its conjugated base, pK_a* values were used instead of normal pK_a (see Supporting Information).

RESULTS AND DISCUSSION

TR-CIDNP measurements were based on a reversible photocycle that includes formation of guanosyl radicals in the quenching reaction of triplet excited dye, 2,2′-dipyridyl (DP), by guanosine-5′-monophosphate (GMP) and subsequent radical termination with restoration of the initial compounds shown in Chart 1. In the present paper, symbols G(−H)• and G are used to denote the radical and molecule with the neutral purine base, respectively. The symbol $G(-H)^-$ refers to the molecule with negatively charged purine base. Alternatively, "GMP" is used for guanosine-5′-monophosphate. CIDNP effects arising during this photocycle were studied by us in detail previously.[14]

The mechanism of CIDNP formation in reversible photochemical reactions has been described in numerous publications and can be briefly summarized as follows. In the photochemical reaction with the triplet precursor molecule, electron or hydrogen atom transfer results in a triplet spin-correlated radical pair. Recombination can only proceed from the singlet state of the pair. Triplet−singlet conversion of the radical pair provides the possibility for radical recombination. The probability of geminate recombination depends on the efficiency of the triplet−singlet transitions driven by magnetic interactions in the pair: the difference in electronic Zeeman frequencies and electron−nuclear hyperfine interactions. Thus, the probability of geminate recombination, giving rise to geminate CIDNP, depends on the nuclear spin configuration. Radicals that escape the geminate recombination carry nuclear polarization opposite in sign to the geminate one because the total polarization is conserved at short times compared to paramagnetic nuclear spin−lattice relaxation. The kinetic profile of CIDNP depends on the second-order termination rate (and thus on the initial radical concentration), the paramagnetic nuclear relaxation time, and the efficiency of degenerate electron exchange. Termination of the radicals in the bulk leads to transfer of polarization to the diamagnetic state. This transferred polarization is opposite in sign to the geminate one and thus gives rise to the so-called CIDNP cancellation effect. In addition, nuclear polarization is formed in diffusive collisions of noncorrelated radical pairs (F pairs) in the bulk coinciding in sign with geminate CIDNP in the case of a triplet precursor.

The ability of CIDNP to visualize the reduction of a radical is based on the spin-sorting nature of the $S-T_0$ mechanism of CIDNP formation in a high magnetic field.[11,13] As mentioned above, during these $S-T_0$ transitions geminate products and radicals that escape geminate recombination acquire equal polarization of opposite sign. Since in our case geminate recombination and reduction of the escaped guanosyl radicals leads to the same product (guanosine), the guanosine H8 polarization is partially canceled depending on the rate of reduction and time of measurement. For an accurate kinetic analysis the rate of radical formation in the quenching reaction (in our case reaction of triplet DP with GMP) should be much higher than the time resolution of the experiment determined by the duration of the detection RF pulse. This is achieved using the appropriate concentration of quencher, which requires knowledge of the quenching rate constants. In the present study concentrations of GMP were taken in accordance with the earlier measured quenching rate constants.[14]

Figure 1 shows CIDNP spectra obtained during photoreactions of dipyridyl and GMP in the absence (left column) and presence of L-cysteine (right column) at neutral pH. The guanosine H8 CIDNP signal in emission is in accordance with the qualitative CIDNP rules.[22] In the absence of cysteine this signal has approximately the same intensity at two delays, while addition of cysteine leads to a noticeable decrease. Enhancements for the protons of the dye are also observed.

Below, we first describe the CIDNP kinetics in the reversible photoreaction of DP and GMP without cysteine in the pH range from 7 to 11.3. Using these data as reference, reaction of

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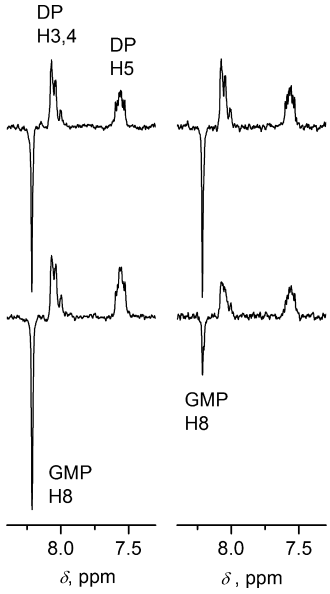

Figure 1. 200 MHz ^{1}H CIDNP spectra obtained from photoreaction between 2,2′-dipyridyl (DP) and guanosine-5′-monophosphate (GMP) in the absence (left column) and presence of 10 mM L-cysteine (right column) at pH* 7.3. Spectra were recorded immediately after the laser pulse (top) and at 100 μs after the laser pulse (bottom).

guanosyl radical with cysteine and its influence on CIDNP kinetics of GMP will be considered in detail.

CIDNP Kinetics in Photoreactions of DP and GMP at Different pH Values. In the pH range studied GMP and its radical have the following pK_a values[23]

$$G \leftrightarrows G(-H)^- + H^+,\ pK_a = 9.4 \quad (2a)$$

$$G(-H)^\bullet \leftrightarrows G(-2H)^{-\bullet} + H^+,\ pK_a = 10.8 \quad (2b)$$

Since pH measurements were performed in D_2O using a H_2O-calibrated pH meter, the readings correspond to so-called pH* values. To take this into account, pK_a* values were used instead of normal pK_a according to the formula pK_a = 0.929pK_a* + 0.42 (ref 21). This gives pK_a* = 9.7 and 11.2 for equilibria 2a and 2b, respectively.

In the reaction of triplet state DP with GMP at 6.8 < pH < 8.4 (6.9 < pH* < 8.6) the neutral guanosyl radical G(−H)$^\bullet$ is formed

$$^TDP + G \rightarrow DPH^\bullet + G(-H)^\bullet \quad (3)$$

with a rate constant $k_q = 1.6 \times 10^8$ $M^{-1}s^{-1}$ (ref 14).

To describe CIDNP kinetics during this reaction an approach is used as suggested by Fischer et al.[24] in which a system of coupled equations for the radical concentration $R(t)$ and the nuclear polarizations of the radicals P_R and the diamagnetic products P is solved

$$R(t) = \frac{R_0}{1 + k_t R_0 t} \quad (4)$$

$$\frac{dP_R}{dt} = -k_t P_R R - k_t \beta R^2 - \frac{P_R}{T_1} - k_{ex} C P_R \quad (5)$$

$$\frac{dP}{dt} = k_t P_R R + k_t \beta R^2 + k_{ex} C P_R \quad (6)$$

Formation of radicals is assumed to be instantaneous on the time scale of the radical reactions. It is controlled using the appropriate concentration of quencher. The value of P is measured in the experiment, R_0 is the initial radical concentration, k_t is the radical termination rate constant, and T_1 is the spin−lattice relaxation time of the polarized nuclei in the radicals. The parameter β represents the polarization per pair, created in F pairs. In the case of a triplet precursor, $\beta = 3P^G/R_0$, where P^G is the geminate polarization at $t = 0$. In accordance with the literature $\beta = 2.8P^G/R_0$ was used.[24,25]

The first terms on the right side of eqs 5 and 6 describe the transfer of polarization from the radicals to the diamagnetic molecules in the termination reaction; the second terms represent formation of polarization in F pairs. The third term in eq 6 corresponds to loss of polarization in the radicals due to nuclear paramagnetic relaxation. The last term in eqs 5 and 6 corresponds to polarization transfer from radicals to parent molecules (with concentration C) in the degenerate electron exchange reaction with rate constant k_{ex}. It was assumed that the yield of radicals that escape from the triplet geminate radical pair is much greater than the yield of geminate recombination and that the radical pair partners disappear only by recombining with one another (with rate constant k_t). The initial polarizations were taken as $P = P^G = -P_R$, which is consistent with the spin-sorting nature of the S−T_0 radical pair mechanism.[11]

The stationary geminate CIDNP level depends on the radical lifetime: the longer the lifetime is the more polarization, opposite in sign to the geminate one, is destroyed by nuclear paramagnetic relaxation.

CIDNP data at neutral pH and simulations are shown in Figure 2a (stars). In the simulations, the relaxation time T_1 = 20 μs for the proton H8 of neutral guanosyl radical G(−H)$^\bullet$ was used.[14] Fitting parameters were the vertical scaling factor and the product of initial radical concentration (R_0) and the second-order radical termination rate constant (k_t). Degenerate electron exchange is not operative at this pH, meaning k_{ex} = 0. The values of $R_0 \times k_t$ for all simulations presented here are given in the Supporting Information.

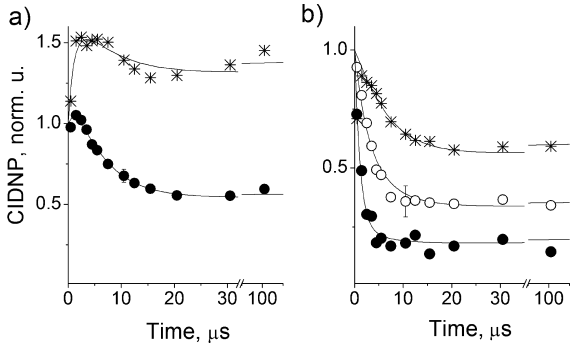

Figure 2. (a) ^{1}H CIDNP kinetics of H8 of GMP, obtained during photoreaction of 8 mM 2,2′-dipyridyl (DP) with 20 mM guanosine-5′-monophosphate (GMP) at pH* 7.5 (stars) and 8 mM DP and 5 mM GMP at pH* 11.3 (circles). (b) ^{1}H CIDNP kinetics of H8 of GMP, obtained during photoreaction of 8 mM DP and GMP at a concentration of 10 (stars), 20 (open circles), and 40 mM (solid circles) at pH* 9.7. pH* values correspond to the readings in D_2O using a H_2O-calibrated pH meter. For all parameters of simulations see text and Supporting Information.

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At pH > 10.4 (pH* > 10.7) the anion of GMP quenches triplet excited dipyridyl via electron transfer with the rate constant $k_q = 1.1 \times 10^9$ M^{-1} s^{-1} (ref 14). Again, the neutral guanosyl radical is formed

$$^{T}DP + G(-H)^- \rightarrow DP^{-\bullet} + G(-H)^{\bullet} \quad (7)$$

This radical is involved in degenerate electron exchange with the parent molecule. In this reaction nuclear polarization (denoted by "P" below) is transferred from the radical to the diamagnetic molecule

$$^{P}G(-H)^{\bullet} + G(-H)^- \rightarrow {}^{P}G(-H) + G(-H)^{\bullet} \quad (8)$$

Since this polarization is opposite in sign to the geminate one, reaction 8 leads to efficient CIDNP cancellation. CIDNP kinetic data at pH 11.3 and simulations are shown in Figure 2a (circles). The rate of reaction 8 is determined by the product of the second-order rate constant of degenerate electron exchange and the concentration of the parent diamagnetic molecules, $k_{ex} \times C$. From our previous measurements the value of the rate constant for degenerate electron exchange is known, $k_{ex} = 4. \times 10^7$ M^{-1} s^{-1} (ref 14).

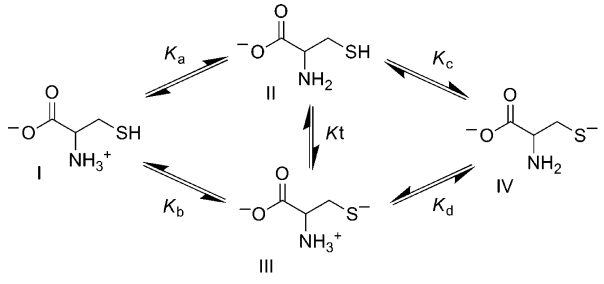
At 8.4 < pH < 10.4 (8.6 < pH* < 10.7), neutral guanosyl radicals are formed either via hydrogen transfer from neutral guanosine or via electron transfer from guanosyl anion. Neutral guanosyl radical has $pK_a = 10.8$ (ref 23). However, as we found out earlier, at pH* 11.3 its deprotonation is negligibly slow in comparison with the lifetime of the radicals and polarization formation on the microsecond time scale of the CIDNP experiment (up to 100 μs).[14]

Thus, in the pH range from ~7 to ~11.3 the conditions for formation of neutral guanosyl radical are fulfilled. Figure 2a indicates that the pH dependence of CIDNP kinetics is determined mainly by the fraction of guanosine molecules ($pK_a* = 9.7$) that are available for degenerate electron exchange with the neutral guanosyl radical. Thus, at pH* 9.7, the CIDNP kinetics detected for three GMP concentrations of 10, 20, and 40 mM and the simulations with $k_{ex} = 4. \times 10^7$ M^{-1} s^{-1} and C equal to one-half the concentration of GMP resulted in a perfect data fit as shown in Figure 2b.

CIDNP Kinetics of GMP in the Presence of Cys and Gly-Cys at Different pH Values. In the pH range from 7.3 to 11.3, cysteine (Cys) has two titratable protons with macroscopic dissociation constants $pK_1 = 8.3$ and $pK_2 = 10.4$ (ref 26). Experimentally determined pK_a's from the pH dependence of the chemical shift are $pK_{a1}* = 8.5 \pm 0.1$ and $pK_{a2}* = 10.8 \pm 0.1$ (see Supporting Information for details) are in good agreement with the calculated[21] values $pK_{a1}* = 8.5$ and $pK_{a2}* = 10.7$.

Macroscopic equilibrium constants are related to microscopic ones K_a, K_b, K_c, and K_d (Scheme 1) as $K_1 = K_a + K_b$, $1/K_2 = 1/K_c + 1/K_d$. Since at pH* 11.3 deprotonation of cysteine is not complete, cysteine-glycine dipeptide with $pK_1* = 7.3$ and $pK_2* = 9.7$ (see Supporting Information) was also used for guanosyl radical reduction. The equilibrium constant for the tautomeric equilibrium between II and III is $pK_t = 0.36$ (ref 26), meaning that cysteine after the first proton release is present for 30% in form II and 70% as III. For Cys-Gly, pK_t is not known from the literature. Results of the kinetic measurements of CIDNP in samples containing the dye, GMP, and cysteine or Gly-Cys at various concentrations are summarized in Figure 3. Kinetic measurement were performed at pH values and with relative concentrations of species I–IV listed in Table 1.

Scheme 1



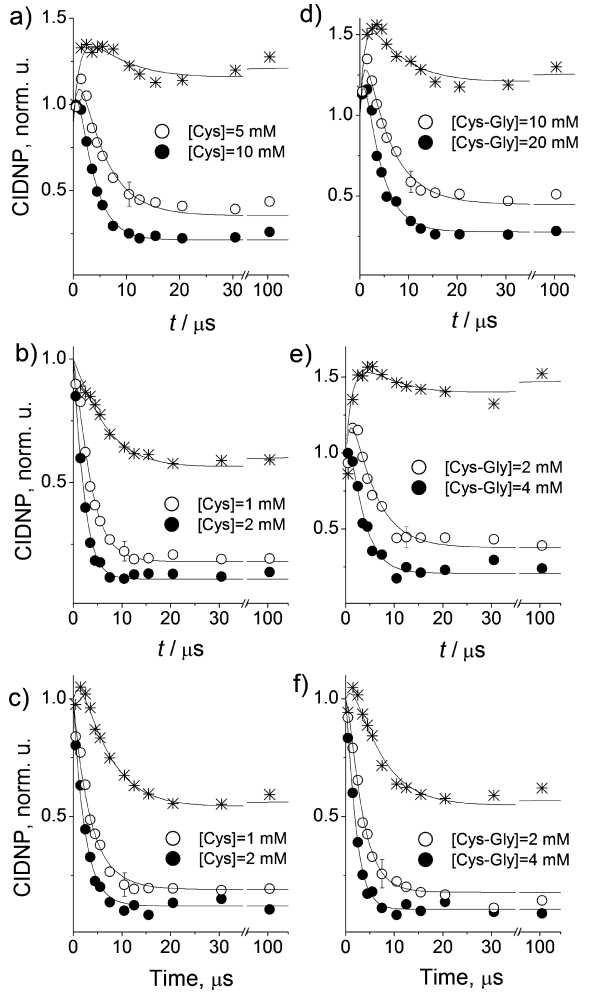


Figure 3. ^{1}H CIDNP kinetics for the H8 proton of guanosine-5′-monophosphate (GMP) obtained during photoreaction between 2,2′-dipyridyl (DP) and GMP (stars) and between DP and GMP in the presence of cysteine (a–c) or cysteine-glycine (d–f) (circles) at pH* 7.3 (a, d), 9.7 (b), 8.5 (e), and 11.3 (c, f). Concentrations (C) of GMP were 20 (a,d), 30 (e), 10 (b), and 5 mM (c, f). pH* values correspond to the readings in D_2O using a H_2O-calibrated pH meter. For all the parameters of simulations see text and Supporting Information.

All kinetic profiles presented in Figure 3 refer to the magnitude of the guanosine H8 signal. It is seen in CIDNP kinetics that addition of cysteine accelerates the decay of CIDNP. The decay rate and stationary CIDNP value depend on the concentration of added cysteine: the higher the concentration of cysteine, the faster is the decay and the lower is the H8 CIDNP stationary value. This observation shows that guanosyl radical is reduced by cysteine. Different

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Table 1. Relative Concentrations of Cysteine and Cysteine-Glycine in Four Ionization States (Scheme 1) and Rate Constants of Guanosyl Radical Reduction at Different pH Values

	pH*[a]	I	II + III[b]	IV	k_r, M^{-1} s^{-1}
Cys	7.3	100%			$(1.9 \pm 0.3) \times 10^7$
	9.7		100%		$(2.0 \pm 0.3) \times 10^8$
	11.3		25%	75%	$(1.7 \pm 0.3) \times 10^8$
Cys-Gly	7.3	50%	50%		$(4.0 \pm 0.6) \times 10^7$
	8.5		100%		$(7.0 \pm 1.0) \times 10^7$
	11.3			100%	$(1.0 \pm 0.2) \times 10^8$

[a]pH* values correspond to the readings in D_2O using a H_2O-calibrated pH meter. [b]For cysteine, II and III are distributed as 30% and 70%. For Cys-Gly, the distribution is unknown.

reaction rates for the various protonation states of Cys and Cys-Gly made it necessary to adjust concentrations of GMP to fall into the kinetic window of the experiment.

When guanosyl radical is reduced by an electron or hydrogen donor the equation for the concentration of guanosyl radical, R_G, no longer coincides with that for the dye radical, R (eq 4), but can be written as

$$R_G(t) = \frac{R_0 e^{-k'_r t}}{1 + k_t R_0 t} \tag{9}$$

The radical reduction reaction is treated as a pseudo-first-order reaction with a rate constant k_r', which is a product of a second-order rate constant and the donor concentration, $k_r \times C_D$. The polarization transfer from quencher radicals to ground state molecules can be described by the following equations

$$\frac{dP_R}{dt} = -k_t P_R R - k_t \beta R R_G - \frac{P_R}{T_1} - k_{ex} C P_R - k'_r P_R \tag{10}$$

$$\frac{dP}{dt} = k_t P_R R + k_t \beta R R_G + k_{ex} C P_R + k'_r P_R \tag{11}$$

Quantitative treatment of the CIDNP kinetics allowed us to obtain the rate constants of reduction, k_r, at different pH* values (Table 1).

It is seen that removal of the thiol proton remarkably increases the efficiency of reduction. The only possible mechanism of guanosyl radical reduction by thiolate anion is electron transfer (eq 1a). The mechanism of reduction by thiol (eq 1b) may be hydrogen transfer, proton-coupled electron transfer, or electron transfer followed by protonation/deprotonation reactions. An order of magnitude lower rate constant for thiol compared to that of thiolate anion may indicate that reaction 1b proceeds via hydrogen transfer. However, our experimental data do not allow one to discriminate between the three possible mechanisms.

From the fractions of cysteine in different ionization states (Table 1) we could obtain the rate constants k_I, k_{III}, and k_{IV} of guanosyl radical reduction by cysteine in the protonation states as given in Scheme 1. If we assume that for tautomerically equilibrated cysteine thiolate is the main particle contributing to the observed rate constant, i.e., $k_{II} \ll k_{III}$, we obtain the rate constants of reduction for different ionization states of Cys as listed in Table 2.

For Cys-Gly, the unknown pK_t makes determination of k_{III} impossible. However, it is seen that the ratio between rate constants for Cys and Cys-Gly in the identical ionization states (I and IV) is approximately the same: 1.9 and 1.6. Thus, the estimate for k_{III} using the same ratio for Cys-Gly is $\sim 1.6 \times 10^8$ M^{-1} s^{-1}. The rate constant k_{II+III} could be considered as the superposition of the rate constants k_{II} and k_{III} with the coefficient equal to the fraction of correspondng ionization states, II and III, which are determined by the equilibrium constant K_t: $k_{II+III} = k_{II} \times K_t/(1 + K_t) + k_{III}/(1 + K_t)$. Since we expect that $k_{II} \ll k_{III}$, $k_{II+III} \approx k_{III}/(1+K_t)$ and we can estimate K_t as 1.3 ($pK_t \approx -0.11$), this means that ~40% of tautomerically equilibrated Cys-Gly is present in the form of thiolate anion. This is consistent with corresponding NMR patterns (see Supporting Information): the NMR spectrum of tautomerically equilibrated Cys-Gly at pH* 8.5 looks different from that of Cys (at pH* 9.60) but similar to the NMR spectrum of Cys at pH* 8.33, where ~30% of the amino acid exists in the form of thiolate anion.

CONCLUSION

Neutral guanosyl radical can be reduced by cysteine, with rate constants close to that obtained previously for tyrosine.[16] The rate constant of guanosyl radical reduction is dependent on the protonation state of cysteine: dissociation of the thiol proton increases the rate constant by an order of magnitude. This is an indication for a switch of the reaction mechanism from hydrogen to electron transfer in accordance with theoretical prediction.[19] The high reaction rate constants of Table 2 indicate that in DNA guanine can be recovered from its neutral radical by cysteine before the radical can participate in harmful biochemical processes.

ASSOCIATED CONTENT

Supporting Information

Parameters of initial second-order radical termination rate used in simulations of CIDNP kinetics obtained during photoreaction of 2,2′-dipyridyl and guanosine-5′-monophosphate in the absence and presence of cysteine or cysteine-glycine; chemical shift titration of L-cysteine; chemical shift titration of L-cysteine-L-glycine. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

Table 2. Rate Constants of Neutral Guanosyl Radical Reduction by Cysteine and Cysteine-Glycine in Different Ionization States (Scheme 1)

	k_I, M^{-1} s^{-1}	k_{III}, M^{-1} s^{-1}	k_{IV}, M^{-1} s^{-1}
Cys	$(1.9 \pm 0.3) \times 10^7$	$(2.8 \pm 0.3) \times 10^8$	$(1.6 \pm 0.3) \times 10^8$
Cys-Gly	$(1.0 \pm 0.6) \times 10^7$	n/a[a]	$(1.0 \pm 0.2) \times 10^8$

[a]For Cys-Gly, only $k_{II} + k_{III}$ is known.

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