Time-Resolved Chemically Induced Dynamic Nuclear Polarization Studies of Structure and Reactivity of Methionine Radical Cations in Aqueous Solution as a Function of pH

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Using time-resolved chemically induced dynamic nuclear polarization (CIDNP) techniques, we have studied the mechanism of the photoreactions of triplet excited 4-carboxybenzophenone (CBP) with L-methionine (Met) and 3-(methylthio)propylamine (MTPA) in aqueous solution and the details of the formation of CIDNP at pH from 6.7 to 13.6. At a pH below the pK_a of the nitrogen atom of Met, the CIDNP is strongly affected by degenerate electron exchange between the S−S cationic radical dimer and the zwitterionic form of Met with the rate constant $k_{ex} = 3.4 \times 10^8$ s^{-1} providing an exhaustive explanation of the pH dependence of steady-state CIDNP that was previously interpreted as a manifestation of fast interconversion among three different methionine radical species (Goez, M.; Rozwadowski, J. *J. Phys. Chem. A* **1998**, *102*, 7945−7953). By analyzing the polarization of different protons formed in geminate recombination as a function of the pH, we obtained the branching ratio between two reaction pathways for oxidative quenching of ^{T}CBP via electron transfer from the sulfur and nitrogen atoms of Met and MTPA. Nuclear spin−lattice relaxation times were determined in the dimeric cation radical of Met ($T_{1,S} = 8.5$ μs). In the cyclic radical cation of MTPA with a three-electron two-center S−N bond, the estimated paramagnetic relaxation is comparatively slow for all protons. Fast deprotonation of the primary aminium radical cation of MTPA and Met in strongly basic solution takes place on the submicrosecond time scale leading to efficient formation of CIDNP in the neutral aminyl radical.

Introduction

Methionine is one of two amino acids that contain a sulfur atom, and among the 20 amino acid residues normally found in proteins, it is the one that is most readily oxidized via electron transfer from the sulfur atom. Oxidation of methionyl residues in proteins plays an important role during pathological conditions and biological aging.[1] It is associated with long-term harmful biological consequences such as Alzheimer disease[2] or in vivo irreversible protein damage.[3] The methionine, as presented on left-hand side of the Chart 1 with R = COO^-, quenches triplet states of carbonyl-containing water-soluble aromatic molecules (carboxybenzophenones, CBP) at a diffusion-controlled rate with a high yield of the cyclic (**II**) and linear (**V**) radicals.[4]

Another reducing quenching mechanism that potentially could operate in basic conditions and therefore might be important for photochemistry of methionine is the electron transfer from the nonbonding electron pair at the nitrogen to the triplet sensitizer, CBP, leading to the zwitterionic aminium radical **III** (Chart 1). This quenching mechanism was first proposed for photooxidation of methionine and related compounds in 1975[5] and was recently found to be responsible for the main channel of the reacton of 4-carboxybenzophenone with aliphatic amino acids.[6] The evidence for this comes from the emergence of the $CBP^{\bullet-}$ radical anion on the nanosecond time scale after photoexcitation. Although for about 30 years an array of circumstantial evidence for two pH-dependent structures of methionine cation radicals (S−N cyclic and linear S-centered) has been assembled using transient absorption[4,7−18] and magnetic resonance detection,[19−23] the formation of an aminium cation radical of sulfur-containing amino acids has not received much attention so far.[1,3,24] The primary aminium radical is one of the main species involved in the oxidative damage of proteins. Up to now, due to the lack of a chromophore group in the radical **III** that precludes it from detection by transient optical absorption, this reducing mechanism for methionine was not taken into account in numerous publications on the photochemistry of methionine-related compounds.[4,7−18]

Chemically induced dynamic nuclear polarization (CIDNP), combining the analytical potential of high-resolution NMR with high detection sensitivity and selectivity with respect to the hyperfine interaction constants, provides information about the spin density distribution and the chemical structure of the short-lived intermediate radicals. Although only the diamagnetic reaction products are detected, the NMR lines of individual nuclei carry information on the paramagnetic stage encoded in the line intensities. Magnitude and phase differ characteristically from their value at thermal equilibrium because spin polarization is formed in the radical strongly depending on the hyperfine coupling constant of the particular nuclei.[25,26] CIDNP spectroscopy as applied to the study of the reversible electron-transfer

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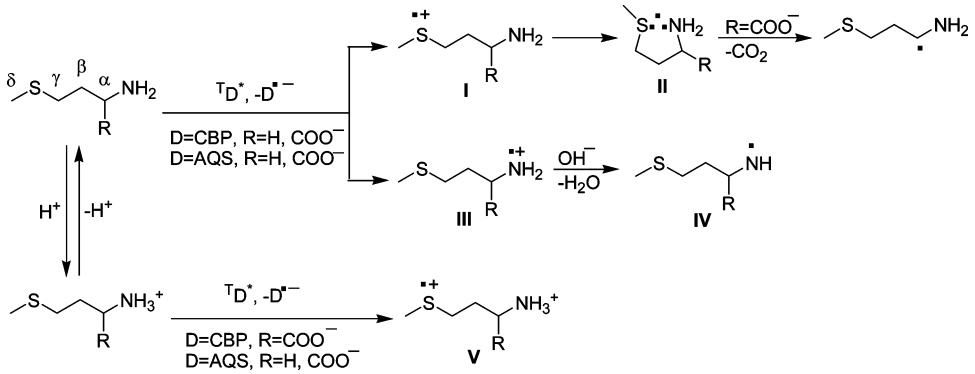
CHART 1



reactions between methionine and 4-carboxybenzophenone in D_2O provided irrefutable evidence for the cyclic structure of the cation radical **II** of methionine formed in basic solution.[21,22] In the cyclic form of the radical **II** with a three-electron two-center bond between sulfur and nitrogen atoms, polarization of all protons in the γ, δ, and α positions of methionine (see Chart 1 for the position labeling) was detected, while in the linear "open form" of radical **V** only the protons in the γ and δ positions were polarized. For radical **III**, only polarization at the α position of methionine is expected, but the reaction pathway leading to formation of radical **III** was not taken into account in previous CIDNP studies.[21,22] From the dependence of CIDNP on the external magnetic field over the wide range from 0 to 7 T, the magnetic resonance parameters (g-factors and hyperfine coupling constants) of radicals **II** and **V** of methionine were obtained.[21] In work reported in ref 22, the ratio of proton polarization at the α and γ positions of methionine (P_α/P_γ) was measured as a function of the pH in the range between 5.8 and 12.2. This dependence showed a remarkable deviation from the titration curve of the chemical shift of the polarized protons. For the explanation, the authors proposed the existence of fast interconversion among three forms (**I**, **II**, and **V**) of methionine cation radicals occurring on the nanosecond time scale and involving two linear radicals (protonated **V** and nonprotonated **I** at the nitrogen atom position), and one cyclic radical **II**, whereas radical **III** was not taken into consideration. The excellent agreement as it was stated for the pH dependence of the ratio (P_α/P_γ) obtained from the analysis of stationary CIDNP patterns and compared with data calculated by modifying standard radical pair theory by taking into account the interconversion of radicals in the geminate stage has still to be substantiated.

The measurement of CIDNP effects in a time-resolved way is an established method, which combines pulsed laser excitation with synchronous pulse NMR detection and stepwise variation of the delay between the laser and NMR detection pulses.[27,28] It provides quantitative information about the kinetics of radical reactions with microsecond time resolution and allows conclusions on the reaction mechanism. In an ongoing project, we applied the time-resolved CIDNP technique to study the mechanism and kinetics of nuclear polarization in reversible reactions of the amino acids tryptophan,[29] tyrosine,[30] and histidine[31] of small peptides[32−34] and several proteins[32,35,36] in aqueous solution. In particular, we showed that the reaction of degenerate electron exchange between radicals of the amino acid and the diamagnetic species leads to efficient transfer of nuclear polarization from the radical to the diamagnetic molecule and strongly affects the CIDNP kinetics leading to the fast decay of polarization. For all three amino acids studied so far, the exchange was found to be important in the regime of pH when the structure of radical and diamagnetic molecule differs only by an electron. In aqueous neutral and basic solution, the carboxyl group of methionine carries a negative charge (pK_a = 2.28), and the amino acid exists in zwitterionic form with a positive charge on the protonated nitrogen (H-Met) below pK_a or with the lone electron pair at the nitrogen above pK_a (see Chart 1). For methionine, the condition for degenerate exchange between radical **V** or its dimer with the diamagnetic molecule is expected to be met at a pH below the pK_a = 9.6. Although methionine monoradicals form intermolecular S−S bonded dimers with the diamagnetic molecules,[20] degenerate electron exchange between ion radicals and diamagnetic species might also in this case affect the kinetics of nuclear polarization. This exchange reaction is not suitable for optical detection because it does not lead to any change in the absorption spectrum, but it is well suited for investigation by time-resolved CIDNP techniques. On the other hand, CIDNP effects reflect dynamic processes in the course of chemical reactions; therefore the analysis of CIDNP patterns obtained solely at steady-state conditions may be rather misleading. Therefore in the present study, we reinvestigate CIDNP by using a pulsed version of the technique, TR-CIDNP, with microsecond resolution and analyze the CIDNP kinetics in the photoreaction of methionine with 4-carboxybenzophenone in aqueous solution at a wide range of pH with the aim to obtain quantitative information about the branching ratio between different types of cation radicals of methionine so formed. For a comparative study, a prototype of methionine, the 3-(methylthio)propylamine, having similar reactivity in oxidative quenching of CBP but with the important advantage that the corresponding radicals so formed are not exposed to the decarboxylation reaction, was chosen to clarify the peculiarities of polarization formation in radicals **II** of methionine that are obscured by the influence of fast decarboxylation. The structures of this compound correspond to R = H on the left-hand side of Chart 1, while the structures with R = COO^- refer to methionine.

Experimental Section

A detailed description of our TR-CIDNP setup was given earlier.[31] The samples, sealed in a standard 5 mm NMR Pyrex ampule, were irradiated by a COMPEX Lambda Physik 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 consisting of a quartz lens, a prism, and a cylindrical light guide (5 mm diameter). TR-CIDNP experiments were carried out with the usual pulse sequence: radio frequency (RF) saturation pulses, laser pulse, evolution time τ, RF detection pulse, free induction decay. Because the background signals in the spectrum origi-

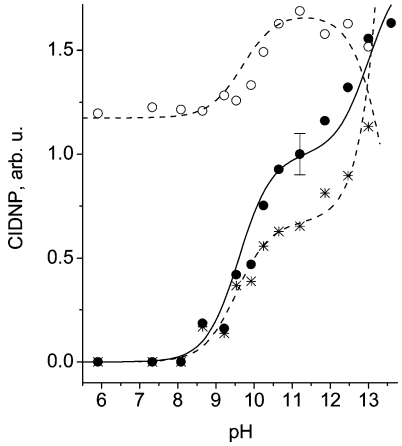


Figure 1. pH dependence of ^{1}H CIDNP obtained in photoreactions of L-methionine and 4-carboxybenzophenone immediately after the laser pulse for the α proton of L-methionine (●), for the γ protons of L-methionine (○), and for the CIDNP ratio of α and γ protons (∗). The solid line shows simulation according to eq 2 with $p_1 = 0$, $p_2 = 1.9$, and $q = 10$.

nating from Boltzmann polarization are suppressed by the saturating pulses, only resonances from the polarized products formed during the variable delay τ appear in the CIDNP spectra. In all kinetic measurements, a RF pulse with duration of 1 μs was used for detection. The timing corresponds to the center of the RF pulse (i.e., 0.5 μs for $\tau = 0$) on all CIDNP plots in Figures 4, 5, and 8.

To avoid the problem of sample depletion, one sample was used to acquire two or four scans for each τ-value. The number of points in a single CIDNP time dependence (8−13) was chosen to provide less than 30% depletion during irradiation of one sample. Depending on the CIDNP enhancement factor, 8−32 samples were used to complete one full CIDNP time evolution with an appropriate signal-to-noise ratio, so every data point in Figures 6−8 represents 32−64 signal accumulations.

L-Methionine (Met), 3-(methylthio)propylamine (MTPA), 4-carboxybenzophenone (CBP), DCl, NaOD, and D_2O were used as received from Sigma-Aldrich with the highest purity available. 9,10-Anthraquinone-2-sulfonate sodium salt (AQS) (97%, Sigma) was recrystallized from ethanol/H_2O before use. Buffer solution was prepared using 0.02 M of NaD_2PO_4. Concentrations were 1.2 mM for CBP, 0.7 mM for AQS, 3 mM for Met, and 3 mM for MTPA, unless otherwise stated. The pH was adjusted by addition of DCl and NaOD. No correction for the deuterium isotope effect on the pH was made.

The CIDNP intensities obtained for the α protons of Met at pH 11.3 and of MTPA at pH 12.0 at $\tau = 0$ were taken as unity; the other experimental points in the Figures 1, 4, 5, 6, and 8 were rescaled accordingly.

Results and Discussion

The pH dependence of CIDNP detected immediately after the laser pulse for the α and γ protons of Met in the photoreaction with CBP is shown in Figure 1. In full accordance with previous results,[21,22] at pH below the pK_a of Met (9.6), polarization is observed for the γ and δ protons only, whereas at pH above pK_a, the α proton is also polarized.

This is illustrated in Figures 2 and 3, where the CIDNP spectra obtained for Met with CBP at pH 13.3 and 6.7, respectively, are shown together with the corresponding dark spectra. This observation, which is only qualitatively in accordance with the observation by Goez and Rozwadowski,[22] can be seen as evidence for the formation of the cyclic structure

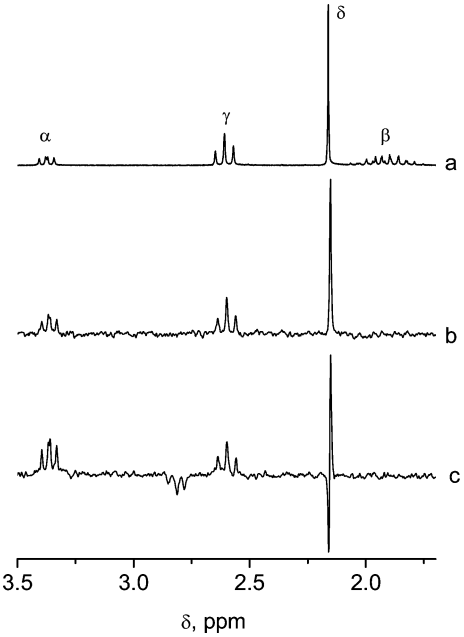


Figure 2. ^{1}H NMR spectrum of L-methionine at pH 13.3 (a) and ^{1}H CIDNP spectra obtained at pH 13.3 in photoreactions of L-methionine and 4-carboxybenzophenone immediately after the laser pulse (b) and with a delay of 100 μs after the laser pulse (c). The NMR spectrum is scaled independently, while the two CIDNP spectra have the same scaling.

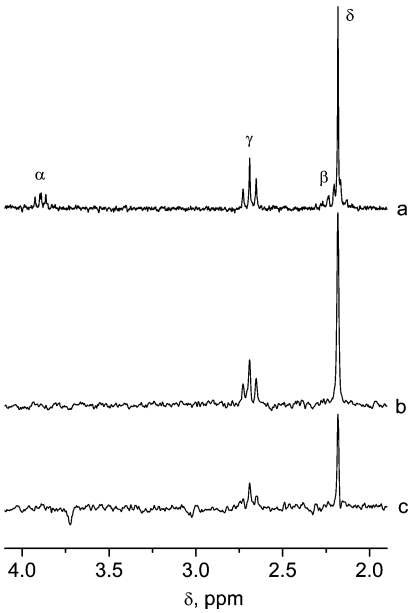


Figure 3. ^{1}H NMR spectrum of L-methionine at pH 6.7 (a) and ^{1}H CIDNP spectra obtained at pH 6.7 in the photoreaction of L-methionine and 4-carboxybenzophenone immediately after the laser pulse (b) and with a delay of 100 μs after the laser pulse (c). The NMR spectrum is scaled independently, while the two CIDNP spectra have the same scaling.

of the cation radical with a five-membered ring. The geminate polarization (obtained at $\tau = 0$) contains signal contributions only from reactants formed in the reversible electron-transfer reaction. In the CIDNP spectrum taken 100 μs after the laser pulse at pH 13.3, additional emissive lines of side products are present. These emissive signals were assigned in ref 37 to γ (triplet, 2.8 ppm) and δ (singlet, 2.2 ppm) protons from the CH_2 and CH_3 groups bonded to the sulfur atom of the aldehyde $O{=}CH{-}CH_2{-}CH_2{-}S{-}CH_3$. Since the α-aminoalkyl radical ($H_2N{-}^{\bullet}CH{-}CH_2{-}CH_2{-}S{-}CH_3$) is formed as the result of

decarboxylation of the methionine cation radical **II** escaped from geminate recombination, the polarization of protons in γ and δ positions is emissive in accordance with spin-sorting $S-T_0$ mechanism of polarization formation in high magnetic field.[25,38] After hydrogen atom abstraction from α-aminoalkyl radicals by 4-carboxy-benzophenone in the ground state, this negative polarization is transferred to the $HN{=}CH-CH_2-CH_2-S-CH_3$. This thioimine is a main product of secondary reactions of α-aminoalkyl radicals with benzophenone, and it is converted to the aldehyde $O{=}CH-CH_2-CH_2-S-CH_3$ via fast reaction with water.[37]

At pH 13.3, in the spectrum taken with a delay of 100 μs after the laser pulse, the intensity of the α proton of methionine is much larger than that in the geminate spectrum, while the growing of the intensity of the γ protons is not detected. This observation is not in accordance with the data obtained in ref 22, where the measurements were made at pH below 12.2 under steady-state irradiation conditions. Moreover, it contradicts the key assumption made in ref 22 that polarization of methionine is formed solely at the geminate stage of the reaction and allows one to suggest that in strong basic solution the polarization of the α and γ protons does not exclusively originate from the same radicals.

In neutral solution, methionine exists in a zwitterionic form with a positive charge on the protonated nitrogen atom, which prevents formation of the cyclic form of the cation radical. In the CIDNP spectrum in Figure 3 taken with no delay after the laser pulse, the nuclear polarization of only the protons in γ and δ positions of the amino acid is found. The absolute intensity of all signals that belong to methionine is much lower in the spectrum taken at 100 μs after the laser pulse than in that formed in the geminate recombination as shown in the spectrum taken with zero delay.

To allow quantitative comparison with the data reported by Goez and Rozwadowski,[22] the pH dependence of P_α/P_γ (geminate polarization ratio of α and γ protons) of Met is shown in Figure 1 by stars. This dependence differs significantly from the corresponding curve found in Figure 3 of ref 22. First, no deviation from the titration curve with $pK_a = 9.6$ is seen. Second, at pH above 11.5, an increase of α proton CIDNP and a slight decrease of polarization of the γ protons is detected, altogether leading to the increase of the ratio of polarizations in contrast to ref 22. As a side remark, we would like to point out that data obtained around pH 9.5 are fault-prone and thus should be disregarded. The reason is the overlapping of the signal of the α proton with the emissive signal of a side product of the reaction, whose intensity is pH dependent. As a result, the determination of P_α/P_γ is already dubious in the pulsed time-resolved mode of CIDNP detection, and the data are therefore omitted in our study, whereas the quantitative analysis by the so-called "background free" steady-state detection of CIDNP as applied in ref 22 is even more disputable.

To clarify the origin of the effects that are responsible for CIDNP generation in the system under study, we performed kinetic measurements at pH 6.7, 11.3, and 13.3. The results of the measurements are shown in Figures 4 and 5. At a pH above 9.6, the signal of the δ protons is overlapping with an emissive signal, and the enhanced absorption of the secondary product superimposes the low-field component of the signal of the γ protons (Figure 3, trace c). Thus, at pH 11.3 and 13.3, only the signal of the α proton was evaluated.

The kinetic curves obtained at pH 6.7 (Figure 4) show a fast decay of CIDNP in time. The decay rate becomes faster when the concentration of the amino acid is increased. This behavior

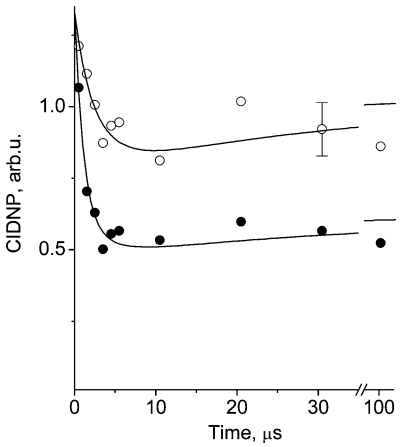


Figure 4. ^{1}H CIDNP kinetics of the photoreaction between L-methionine and 4-carboxybenzophenone observed for the γ protons at a pH of 6.7 and a concentration of L-methionine of 1.1 mM (○) and 2.2 mM (●). The lines were calculated according to the procedure described in the text. For the values of the parameters used, see the text.

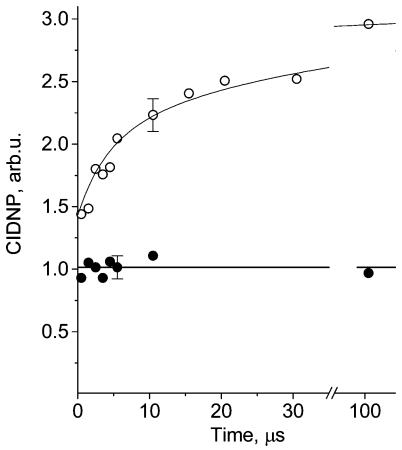


Figure 5. ^{1}H CIDNP kinetics of the photoreaction between L-methionine and 4-carboxybenzophenone observed for the α proton at pH 11.3 (●) and pH 13.3 (○). The lines were calculated according to the procedure described in the text. For the values of the parameters used, see the text.

is typical for degenerate electron exchange, and we take it as clear evidence that this process takes place in the bulk. By transient optical experiments, the absorbance of the dimeric sulfur radical cations with the maximum at 408 nm is well characterized.[4] Also in time-resolved EPR (CIDEP) spectra observed for the photoreaction of triplet 9,10-anthraquinone-2-sulfonic acid (AQS) with methionine in neutral aqueous solution,[20] the dimeric sulfur radical cations were detected with hyperfine splitting corresponding to six and four protons and assigned to the δ and γ protons ($a_\delta = 0.712$ mT, $a_\gamma = 0.566$ mT). On the basis of these data, we attribute the fast decay of the CIDNP signal to the following degenerate exchange reaction:

$$*[H_3C(R)S\therefore^{+}S(R)CH_3] + S(R)CH_3 \xrightarrow{k_{ex}} *H_3C(R)S + [H_3C(R)S\therefore^{+}S(R)CH_3] \quad (1)$$

Here $H_3C(R)S$ denotes the methionine molecule, and the asterisk denotes dynamic nuclear polarization. TR-CIDNP opens the opportunity to study the degenerate exchange process between species in their radical state (or dimeric radical cations) and their diamagnetic ground state. Since the spin polarization originates from radicals that escaped from the geminate cage,

it is opposite in sign to the polarization of the geminate products; therefore this exchange reaction leads to efficient cancellation of the geminate polarization as is observed for the γ and δ protons of methionine. The numerical simulation of the CIDNP kinetics is shown in Figure 4 by the solid line and, as it will be explained further below, is done by including the term $k_{ex}CP(R)$ that describes the transfer of radical polarization, $P(R)$, from the paramagnetic to the diamagnetic species with concentration C in the degenerate electron exchange.

We conclude that the degenerate electron exchange that determines the CIDNP kinetics of the methionine protonated at the amino group is responsible for the deviation of the CIDNP pH dependence from the titration curve in ref 22. At a concentration of the starting compound of 20 mM as used in that work CIDNP measured under steady-state conditions is strongly attenuated by the exchange reaction 1. The degree of cancellation is determined by the concentration of the parent exchanging molecule and is therefore pH-dependent: at a pH well below pK_a (9.6), 100% of methionine molecules can participate in the exchange reaction, whereas only 50% are available at pH 9.6. Thus, as the pH decreases, the stationary CIDNP value of γ and δ protons formed in the linear form of methionine decreases; hence the ratio of stationary polarizations of α and γ protons of Met increases beyond the value expected for the normal titration curve. No interconversion among three forms of methionine radical cations is needed to explain the observed stationary CIDNP pH dependence. An additional argument against the scheme introduced in ref 22 is the pK_a value of the amino group of the linear methionine cation radical, which is stated to be 8.5.[22] The characteristic time τ_d of deprotonation can be expressed via the ionization constant K_a and the protonation rate constant k_p ($\sim 10^{10}$ M^{-1} s^{-1}): $\tau_d = K_a^{-1}k_p^{-1}$, which is 30 ms at p$K_a$ = 8.5. This time constant is too big to have equilibrium conditions established during the lifetime of the radicals, which under the conditions of CIDNP experiments can hardly be longer than 0.1 ms.

At a pH of 11.3, the CIDNP of the α proton of Met is constant in time (Figure 5, solid symbols), in full accordance with the fast decarboxylation rate reported for the cyclic Met radical (5×10^6 s^{-1}),[39] which allows only geminate polarization to be formed. A remarkably growing CIDNP kinetics is obtained at pH 13.3 (Figure 5, open symbols). At pH values between 11.3 and 13.3, a monotonic increase of CIDNP at 100 μs relative to that at zero delay with a growing intensity of the geminate polarization is observed (not shown).

To gain more insight into the radical reactions of methionine that lead to the observed CIDNP effects, we explored the ^{1}H CIDNP in the reaction of the prototype of methionine, 3-(methylthio)propylamine (MTPA), which does not contain the carboxyl group (R = H in Chart 1) and therefore has a lifetime of its cyclic radical **II** that is not limited by the fast decarboxylation reaction. MTPA is commonly used as a model compound for unraveling the redox chemistry in sulfur-containing amino acids and peptides; therefore the kinetics and structural properties of the cyclic radical **II** formed from MPTA in aqueous solution are well characterized.[40] The pH dependence of geminate CIDNP detected in photoreactions of MTPA with different dyes is shown in Figure 6; for two different pH values, the CIDNP spectra taken without time delay between laser pulse and NMR pulse are presented in Figure 7. The CIDNP patterns at zero delay closely resemble in all their peculiarities those of methionine: polarization is detected for the α, γ, and δ protons but not for the β protons. No polarization is detected for MTPA with CBP below the pK_a of the amino group, 10.5,[40] indicating

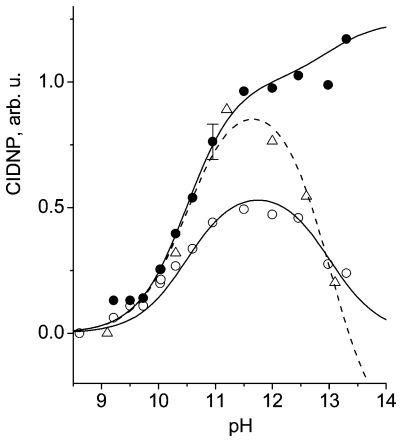


Figure 6. pH dependence of ^{1}H CIDNP obtained in the photoreaction of MTPA and 4-carboxybenzophenone immediately after the laser pulse for the α protons of MTPA (●) and for the γ protons of MTPA (○) and in the photoreaction of MTPA and 9,10-anthraquinone-2-sulfonate sodium salt immediately after the laser pulse for the α protons of MTPA (△). The lines were simulated according to eq 2 with the following parameters: $p_1 = 0$, $p_2 = 0$, and $q = 10$ (fitting to ○); $p_1 = 0$, $p_2 = 1.2$, and $q = 10$ (fitting to ●); $p_1 = 0$, $p_2 = -0.45$, and $q = 10$ (fitting to △).

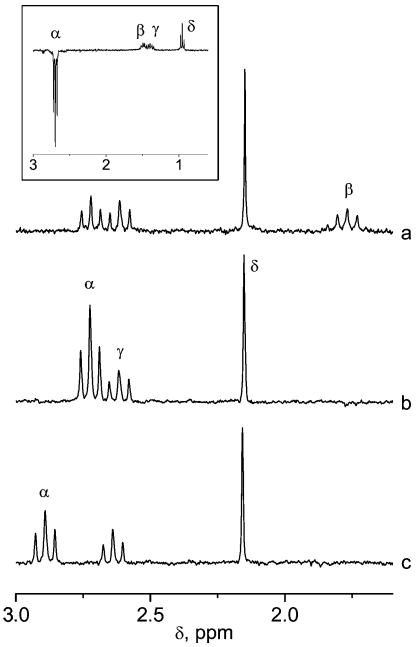


Figure 7. ^{1}H NMR spectrum of MTPA at pH 12.6 (a) and ^{1}H CIDNP spectra obtained in the photoreaction of MTPA and 4-carboxybenzophenone immediately after the laser pulse at pH 12.6 (b) and 11.0 (c). NMR spectrum and CIDNP spectra are scaled independently. The insert shows the ^{1}H NMR spectrum obtained during light irradiation of the solution containing 3 mM of *N*-butylamine and 0.5 mM of AQS, pH 12.2.

that no reaction takes place between the triplet excited CBP and protonated MTPA. Polarization of both α and γ protons first grows with increasing pH with pK_a = 10.5, and at higher pH, the direction of CIDNP amplitude change for these two types of protons becomes opposite, similar to that obtained for Met. We also checked the CIDNP of MTPA in the reaction with another sensitizer, 9,10-anthraquinone-2-sulfonate (AQS), that was used before in a time-resolved CIDEP study of methionine cation radicals.[20] In the photoreaction with AQS, CIDNP of MTPA was found in the whole pH range. The pH dependence of the geminate CIDNP amplitude for the α protons is shown in Figure 6 by triangles. It is seen that after passing

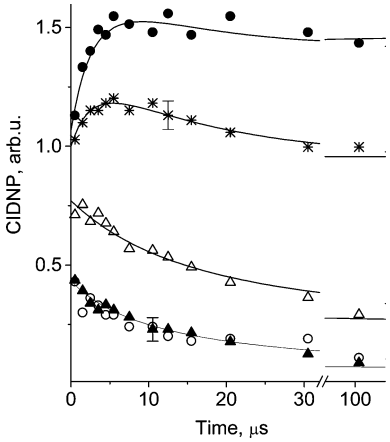


Figure 8. ^{1}H CIDNP kinetics obtained in photoreactions of MTPA and 4-carboxybenzophenone for α protons at pH 12.6 (●), pH 12.0 (∗), and pH 11.0 (△) and for γ protons at pH 12.6 (○) and pH 11.0 (▲). The lines were calculated according to the procedure described in the text. For the values of the parameters, see the text.

through a maximum at around pH = 11.5 the CIDNP of the α protons decreases at higher pH. The corresponding pH dependence for the γ protons (not shown) starts from a nonzero value below pK_a and is qualitatively the same as that obtained for Met and CBP (cf. Figure 1).

The CIDNP kinetics obtained at different pH for the α and γ protons (Figure 8) has the following peculiarities. A monotonic decay with a pH-independent decay rate is detected for the γ protons, while the influence of pH is seen in the attenuation of the geminate CIDNP amplitude in accordance with the pH dependence in Figure 6 (open circles). In Figure 8, an almost coinciding kinetics obtained at pH 11.0 and 12.6 is shown by solid triangles and open circles, respectively. Furthermore, the kinetics of the γ protons is independent of the concentration of MTPA. For the α protons, the CIDNP kinetics changes from a monotonic decay at pH 11 to an initial rise followed by a slight decrease at pH 12 and 12.6.

That the kinetics of the γ protons stays invariant provides as conclusion that only one sort of species is responsible for the formation of CIDNP for these protons. Based on the previous studies, we conclude that this species is the cyclic cation radical **II**, which is formed very rapidly from the linear S-centered cation radical **I**. The kinetics of the γ protons seen here differs significantly from that detected earlier in reversible radical reactions, which is usually characterized by an initial growth and subsequent decay to a stationary value. Since the CIDNP kinetics is not affected by the variation of concentration of MTPA, we conclude that the degenerate electron exchange is not operative for the cyclic cation radical. This result supports our hypothesis about the low efficiency of CIDNP formation in the bulk for radical pairs containing the cyclic radical **II**. This hypothesis is based on the assumption of fast conformational changes that modulate the magnitude and the sign of hyperfine interaction constants in radical **II** of MTPA. This hypothesis is supported by the ESR spectrum of MTPA, which becomes wide and structureless at pH ≈ 10.5 (Yashiro, White, Yurkovskaya, Forbes, in preparation for *Molecular Physics*).

The CIDNP kinetics for the α protons of MTPA gives evidence for CIDNP formation from multiple sources. The opposite behavior in CIDNP amplitude change for the α protons obtained in reactions with different dyes (Figure 6) suggests that the aminium cation radical **III** is formed in the system under study. According to Kaptein's rules,[38] the sign of the geminate polarization of a given nucleus for the case of a triplet precursor is determined by the sign of $\Gamma = (g_1 - g_2)A$, where A is the hyperfine coupling constant of that nucleus and $g_{1,2}$ denote the g-factor of the radicals in the pair, with the indices 1 and 2 referring to the radical containing the given nucleus and the partner radical, respectively. The g-factors of the radical species are $g(NR_2^{\bullet+}) = 2.0035$,[41] $g(CBP^{\bullet-}) = 2.0033$,[42,43] and $g(AQS^{\bullet-}) = 2.0039$;[44] therefore, $\Delta g = (g_1 - g_2)$ for the aminium radical has the opposite sign in the pairs with radicals originating from CBP as compared to AQS. Thus enhanced absorption is formed for the α protons in the pair with $CBP^{\bullet-}$ and emission in the pair with $AQS^{\bullet-}$. To check this argument, we detected the NMR spectrum during light irradiation of an aqueous solution containing *N*-butylamine and AQS at pH 12.2. It is seen in the insert of Figure 7 that under the light irradiation the α protons are negatively polarized, while the β, γ, and δ protons do not carry any dynamic polarization but have the same intensity as in the thermally polarized NMR spectrum without irradiation (not shown). This observation is in accordance with the spin density distribution as it is expected for the primary aminium cation radical.

The aminium radical cation **III** does not contribute to the CIDNP of the γ protons of MTPA. The fact that the polarization of these protons drops for pH > 11.5 provides further evidence that there are two competitive channels of the oxidative quenching reaction, one being the formation of the S-centered cation radical **I** followed by its fast cyclization to radical **II** and the other being electron transfer from nitrogen with aminium radical cation formation. The efficiency of the second channel is pH-dependent. The aminium cation radical **III** does not form the cyclic species **II**; otherwise, the geminate polarization of γ protons would not decrease with pH.

We simulated the dependence of the geminate CIDNP on pH obtained for protons of the two compounds, methionine and MTPA, using the following equation:

$$P = p_1\frac{10^{-\mathrm{pH}}}{10^{-\mathrm{p}K_a} + 10^{-\mathrm{pH}}} + \frac{10^{-\mathrm{p}K_a}}{10^{-\mathrm{p}K_a} + 10^{-\mathrm{pH}}} \times \left(\frac{1}{1 + q \times 10^{\mathrm{pH}-14}} + p_2\frac{q \times 10^{\mathrm{pH}-14}}{1 + q \times 10^{\mathrm{pH}-14}}\right) \quad (2)$$

Here, the first term describes CIDNP originating from the molecule with a charged amino group, while CIDNP from its conjugated base is associated with polarization described by the second term. In the parentheses, the first and the second term reflect the shared contribution to CIDNP from cyclic (**II**) and aminium (**III**) radicals, respectively. The factor q determines the relative contributions of the two above-mentioned channels of quenching at a given pH. The CIDNP enhancement factor for the cyclic radical was taken as a unity; p_1 and p_2 are the enhancement factors for the linear S-centered radical **V** and the aminium radical **III** relative to the cyclic radical, respectively.

For the γ protons of MTPA (open circles in Figure 6), p_1 and p_2 were taken as zero; the best fit for the pH dependence was obtained at $q = 10$ (the second fitting parameter was the scaling factor). This value is in a good agreement with the ratio of rate constants obtained for the quenching of 4-carboxybenzophenone by methionine[4,5] ($k_q = 1.6 \times 10^9$ M^{-1} s^{-1}) and alanine[5,6] ($k_q = 1.6 \times 10^8$ M^{-1} s^{-1}) at pH 12. For the α protons of MTPA with CBP, we used $p_1 = 0$ and $q = 10$ obtained from simulation of the γ protons, and the best fit was obtained at $p_2 = 1.2$. With AQS as photosensitizer, $p_2 = -0.5$ was used. In a similar way, the pH dependence of CIDNP for the α proton of Met was simulated (Figure 1). The following parameters for methionine were found from the simulations: $p_1 = 0$, $p_2 = 1.9$,

and $q = 10$. The dashed lines in Figure 1 do not represent the simulation, because the experimental data for the γ protons are distorted by the overlapping of the side-product signal in the spectrum.

From the CIDNP kinetic measurements, it follows that the aminium radical **III** formed as a result of oxidative quenching from nitrogen undergoes fast deprotonation to form the neutral aminyl radical **IV**; otherwise degenerate electron exchange between the aminium cation radical and the neutral MTPA would lead to a decay of CIDNP kinetics, in contrast to our observation. The pK_a value reported for the corresponding aminium radical derived from glycine is 2.6[45] giving an estimate of 40 ns for the characteristic time of deprotonation, a value that is also in agreement with our CIDNP observations.

Thus the collection of the results gives strong evidence for the reaction scheme shown in Chart 1.

To describe the CIDNP kinetics, we used the set of differential equations following from the approach suggested by Fischer[46] and modified it in accordance with the peculiarities of the system under study.

$$R_1(t) = \frac{R_0}{1 + k_t R_0 t} \tag{3}$$

$$R(t) = \frac{e^{-k_d t} R_0}{1 + k_t R_0 t} \tag{4}$$

$$\frac{dP(R)}{dt} = -k_t P(R) R - k_0 \beta R R_1 - \frac{P(R)}{T_1} - k_{ex} C P(R) - k_d P(R) \tag{5}$$

$$\frac{dP(Pr)}{dt} = k_t P(R) R + k_0 \beta R R_1 + k_{ex} C P(R) \tag{6}$$

Here R_0 is the initial radical pair concentration, $R_1(t)$ the concentration of anion radicals of the dye, $R(t)$ is the concentration of methionine radicals, $P(R)$ is the polarization of the radicals, $P(Pr)$ is the polarization of the diamagnetic products, k_t is the rate constant of radical termination, T_1 is the nuclear paramagnetic relaxation time, the term $k_{ex}CP(R)$ describes the transfer of polarization from paramagnetic to diamagnetic species with concentration C in the degenerate electron exchange, and k_d is the decarboxylation rate constant. Parameter β denotes the polarization per radical pair created in so-called F-pairs. It is related to the geminate polarization P^G via the quantity γ, which denotes the ratio of polarization created in F pairs to the geminate polarization; $\beta = \gamma P^G/R_0$.[46] For a triplet precursor, $\gamma = 3$.

The CIDNP kinetics of the protons of MTPA at pH 11 that is formed only in the radical with the cyclic structure was simulated using the following parameters: $R_0k_t = 1.0 \times 10^5$ s^{-1}; $k_d = 0$; $k_{ex} = 0$; α protons − $T_{1,c} = 600$ μs; $\gamma_c = 0.2$; γ protons − $T_{1,c} = 150$ μs; $\gamma_c = 0.6$ (here, subscript "c" denotes attribute of the cyclic structure). The kinetics of the α protons at higher pH is treated as a superposition of two contributions, one originating from the cyclic structure with the weight χ and the other with the weight $(1 - \chi)$, from the linear N-centered radical, being aminium **III** at the geminate stage and subsequently converted into aminyl radical **IV**. The different contributions of the radicals to CIDNP formation at the geminate and homogeneous stage is taken into account by adjusting the parameter γ to a value different from 3. The parameters for the nuclear polarization formed in the cyclic radical ($T_{1,c}$, γ_c) were taken from the fit at pH 11. The value χ at a given pH was calculated as $1/(1 + q \times 10^{pH-14})$ with the q value obtained from the best fit of the dependence of geminate CIDNP on pH by eq 2. The parameter R_0k_t was increased by 30% up to 1.3×10^5 s^{-1} since at pH 11 only 76% of MTPA molecules are deprotonated at the amino group and contribute to the quenching reaction, making it less efficient than at that pH 12 and 12.6 where almost 100% of MTPA molecules participate in the quenching reaction, which would result in an increased radical pair concentration. The following parameters correspond to the best-fit simulation shown in Figure 8: $T_{1,N} = 25$ μs; pH 12, $\gamma_N = 11$, $\chi = 0.91$; pH 12.6, $\gamma_N = 6.4$, $\chi = 0.72$ (here, subscript "N" denotes the common attribute of the radicals **III** and **IV**). The fact that $\gamma_N > 3$ indicates that CIDNP formation is more efficient in the aminyl than in the aminium radical. This is reasonable since the g-factor of the aminyl radical is 2.0049 and thus the term $\Delta g B_0$ at $B_0 = 4.7$ T is closer to the condition of maximum CIDNP in comparison with the aminium radical having $g = 2.0035$.

We were unable to fit the kinetics at pH 12.0 and 12.6 with a single value of γ_N. That probably indicates that deprotonation of aminium radical **III** is accelerated by OH^- and that at pH 12.6 radical displacement already takes place at times <0.5 μs, the lower limit of our time resolution.

We checked the possibility of direct formation of the aminyl radical **IV** via deprotonation of the cyclic form **II** with the rate constant k_{-H} by incorporating the corresponding terms into the set of eqs 3−6. A value of k_{-H} equal to or smaller than 5×10^3 s^{-1} had no influence on the simulation of the CIDNP time dependence. The quality of the fit, however, deteriorated as k_{-H} was increased above 1×10^4 s^{-1}. Thus, we conclude that deprotonation of the cyclic radical **II** does not take place on the time scale of CIDNP formation.

For the α proton of methionine, the cyclic radical **II** gives a constant contribution to the CIDNP kinetics because of the high decarboxylation rate constant prohibiting the cancellation of polarization. For consistency, we simulated the CIDNP kinetics shown in Figure 5 by open symbols using the following parameter set: $T_{1,N} = 13$ μs; $T_{1,c} = 150$ μs; $R_0k_t = 8 \times 10^4$ s^{-1}; $k_d = 5 \times 10^6$ s^{-1}; $\gamma_N = 3$; $\gamma_c = 0.6$; $\chi = 0.33$; $k_{ex} = 0$.

The best fit of the exchange-type kinetics obtained for the γ protons at pH 6.7 with two concentrations of methionine was achieved using eqs 3−6 with the following parameters: $T_{1,S} = 8.5$ μs; $R_0k_t = 1.1 \times 10^5$ s^{-1}; $k_{ex} = 3.4 \times 10^8$ M^{-1} s^{-1}. The difference of the two curves is determined only by the change of parameter C, which is the concentration of the starting compound L-methionine (1.1 mM for open symbols, 2.2 mM for solid symbols in Figure 4). We used the term describing ion-molecular charge-transfer reaction involving dimer radical cation and monomeric diamagnetic molecule in the same form as that for degenerate exchange reaction between radical and molecule according to the threoretical results obtained for low viscosity solution.[47]

Conclusion

The present work describes the results of a comprehensive time-resolved CIDNP study of the reactions of methionine and 3-(methylthio)propylamine with 4-carboxybenzopheone in aqueous solution at different pH (6.7−13.3). Our results give clear evidence that two oxidative quenching mechanisms formally attributed to electron abstraction from sulfur and nitrogen atoms are operative in reactions with excited triplet states of suitable acceptor molecules. After electron transfer from the sulfur atom to the triplet excited benzophenone, two structures of the radical cation having different distributions of spin density are formed.

At pH values above 9.6, the radical cation exists in a cyclic form with a two-center, three-electron bond between the nitrogen and sulfur atoms, while in solutions below pH 9.6, the Met radical cation has a linear structure. The kinetics of the polarization in the 3-(methylthio)propylamine chosen as a model compound to study magnetic resonance properties in cyclic five-membered ring radicals allows us to determine the nuclear paramagnetic relaxation time T_1 for all polarized protons and to show that protons in the vicinity of the nitrogen have a much faster relaxation in the linear form of the radical than in the cyclic one. The TR-CIDNP measurements also allow demonstration of the crucial influence of the degenerate electron exchange between cationic radical dimers and diamagnetic molecules on the intensity of the CIDNP signals in the pH range below the pK_a of the amino group of methionine. The rate constant for the electron exchange was obtained. The comparison of the experimental results with literature data obtained by steady-state CIDNP measurements casts doubt on the hypothesis that interconversion among three types of methionine cation radicals occurs. Instead a competitive channel for oxidative quenching of the triplet sensitizer by direct electron transfer from the nitrogen atom leading to formation of the aminium radical cation was revealed that is operative in strongly basic solution. Our study of the CIDNP kinetics formed in the photoreaction of 3-(methylthio)propylamine and methionine at strongly basic condition shows a much higher efficiency of polarization formation in the aminyl radical than in the two other species, the aminium and cyclic radicals. We anticipate that starting from this detailed investigation of methionine the existence of the channel of oxidative quenching via electron transfer from the nitrogen of the amino group will be shown to be a common feature of other amino acids and proteins. The results presented here demonstrate that the time-resolved CIDNP technique is very suitable for characterizing short-lived paramagnetic reaction intermediates, whereas it should be kept in mind that the use of the continuous wave CIDNP detection mode can lead to misinterpretations concerning the structure and dynamics of elusive radicals.

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