CIDNP Spectroscopic Observation of ($S.^{+}.N$) Radical Cations with a Two-Center Three-Electron Bond During the Photooxidation of Methionine**

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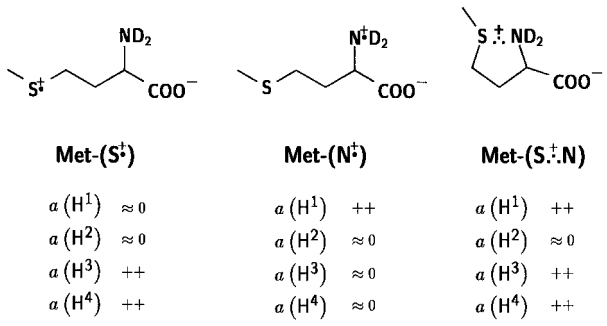
Considerable effort has been devoted to investigating photoinduced[1] and radiation-induced oxidations[2] of sulfur-containing amino acids.[3] A major motivation of these studies has been that the reactions of these residues in proteins are thought to play an important role for long-range electron transfer across cell membranes[4a] and for oxidative damage of cell components.[4b]

The primary step of the sensitized photoreactions is photoinduced electron transfer from the amino acid to the sensitizer;[1] the same radical cations that are formed in this process are also early intermediates in the radiolysis experiments.[2] On the basis of UV/Vis spectroscopic evidence it was concluded[1d,g,2c–e] that in the case of methionine (Met) and related compounds the initial open-chain radical cations stabilize by the formation of cyclic structures Met-($S.^{+}.N$) with an intramolecular two-center three-electron bond between sulfur and nitrogen (Scheme 1[5,6]). Although the



Scheme 1.

($S.^{+}.N$) species are generally accepted intermediates in these reactions, to date there exists virtually no direct structural proof by methods that are sensitive to the distribution of unpaired spin density (i.e., magnetic resonance techniques). The only exception is a solid-state study in which poorly resolved EPR spectra exhibiting only a ^{14}N splitting were obtained during pulse radiolysis of Met in concentrated aqueous solutions of lithium halides below 160 K.[7] Here we report the first magnetic resonance observation in the liquid phase of the ($S.^{+}.N$) species derived from methionine.

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EPR spectroscopy of these radical cations is hampered by the fact that they are very short-lived in liquid solution. Measurements of chemically induced dynamic nuclear polarizations (CIDNP)[8] provide another method to probe the structures and spin-density distributions of radical intermediates that is exactly tailored for this situation. The polarizations are generated by nuclear spin sorting in radical pairs, which in turn is caused by the concomitant action of nuclear spin dependent intersystem crossing (by the interplay of Zeeman and hyperfine interactions) and electron spin selective reactions of the pairs (cage recombination is usually possible in the singlet state only.) Detection is accomplished by recording the NMR spectrum of the diamagnetic reaction products, in which the polarizations manifest themselves as anomalous line intensities.

The efficiency of spin sorting increases with increasing hyperfine coupling constant a_i of the proton or nucleus in question. Hence, the relative polarization intensities of the different protons in the products, the polarization pattern,[9] reflect the relative hyperfine coupling constants in the radicals. As the buildup of the polarizations is complete after the lifetime of the paramagnetic intermediates (nanoseconds or even shorter) but the polarizations persist in the diamagnetic products for the spin-lattice relaxation time T_1 (seconds for protons), the polarization pattern may be regarded as the frozen signature of the intermediate radicals. CIDNP spectroscopy is therefore sensitive to faster processes than is EPR spectroscopy. It is also in some respect more helpful for characterizing radical intermediates, because the CIDNP spectrum immediately establishes a correspondence between a particular value a_i and a particular nucleus i in the products.

CIDNP spectra observed in the photoreactions of methionine with 4-carboxybenzophenone (CB) in D_2O at pH 5.8 and 12.2 are shown in Figure 1. By a special pulse sequence,[11] the NMR signals of all unreactive molecules in the sample were eliminated, and the spectra display pure CIDNP signals only. Protons H_3 and H_4 of Met (i.e., those adjacent to sulfur) bear strong polarizations in both experiments, whereas H^1 (in the α position with respect to nitrogen) is unpolarized at pH 5.8 but strongly polarized at pH 12.2. Referring to equal numbers of protons (two equivalent protons H^3 *vs.* one proton H^1), integration of the signals shows that the ratio of CIDNP intensities $P(H^1)$: $P(H^3)$ is about 1.3 in the limit of high pH. At intermediate pH the polarization pattern lies between the two extremes. All these CIDNP signals appear in absorption. Proton H^2 ($\delta = 1.8...2.0$ ppm, depending on pH) is unpolarized. Owing to deprotonation of the amino function at higher pH (pK_{a2} of Met in D_2O was determined to be 9.65 ± 0.09 by NMR titration), the resonance frequencies of the methionine protons, especially of H^1, shift considerably with pH. However, comparison with the NMR signals in the dark renders the assignment given beyond doubt.

The mechanism relevant for the polarizations of the substrate Met is shown in Scheme 2. The triplet state ^{3}CB of the sensitizer is quenched by electron transfer[1] from Met to give a triplet radical pair $\overline{^{3}CB^{-}\cdot\ Met^{+}\cdot}$. The exact localization of the unpaired spin density in the methionine radical cation $Met^{+}\cdot$ (the three possibilities—the sulfur-centered radical

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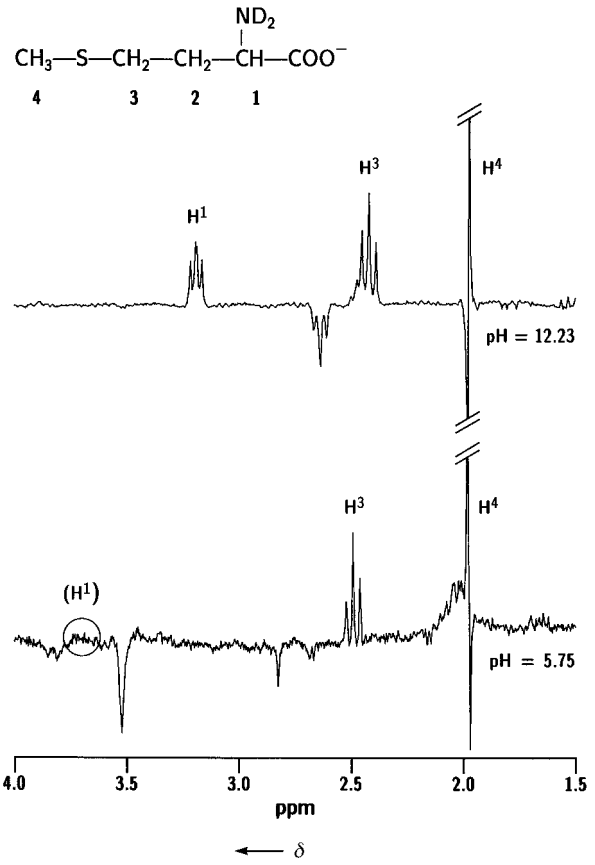
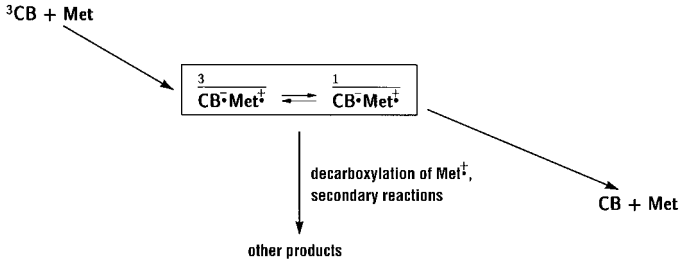


Figure 1. Background-free ^{1}H-CIDNP spectra (250 MHz) obtained in the photoreactions of 4-carboxybenzophenone with methionine in D_2O at pH 5.75 (bottom) and 12.23 (top).[10] Only the spectral region of interest is shown, and only the resonance of the methionine protons (compare the formula at the top)[6] are assigned. The spectra were normalized with respect to the triplet of H^3.



Scheme 2.

cation Met-(S^+), the nitrogen-centered radical cation Met-(N^+), and the species Met-($S^{+}.N$) with the two-center three-electron bond—are displayed in Scheme 1[5,6]) is of no consequence for the basic mechanism of Scheme 2: In any case, back electron transfer is feasible for singlet pairs $^1\overline{CB^-\cdot Met^+}$ only, because the radical pairs are 18 kJ mol^{-1} lower in energy than ^{3}CB[1e] and the triplet energy of the amino acid is even higher than that of the sensitizer.[1f]

Decarboxylation of Met^+ ultimately produces other products, some of them spin-polarized (compare the CIDNP spectra). However, this pathway cannot lead back to Met. Hence, all complications due to the rich secondary chemistry[1d] of the system are avoided by analyzing only the polarizations of the regenerated starting amino acid Met.

Comparison with model compounds[1f]—*S*-methylcysteine, which forms sulfur-centered radical cations only, for Met-(S^+), and alanine, in which the radical cation is necessarily nitrogen-centered, for Met-(N^+)—shows the *g* values of both Met-(S^+) and Met-(N^+) to be larger than that of CB^-. The *g* value of Met-($S^{+}.N$) (see also ref. [7]) must fall between that of Met-(S^+) and Met-(N^+) owing to the increased spin-orbit coupling in the sulfur-containing species Met-($S^{+}.N$) with the two-center three-electron bond compared to Met-(N^+), and is therefore also larger than that of CB^-. For any of the three methionine-derived radical cations, the nonnegligible proton hyperfine coupling constants must be positive because their source is hyperconjugation;[12] for Met-(S^+) and Met-(N^+) this is confirmed by CIDNP experiments[1f] on the above-mentioned model compounds. According to Kaptein's rule,[13] the observed absorption signals for the protons of Met are thus consistent with the reaction mechanism in Scheme 2 (triplet precursor, $\mu = +1$; singlet product, $\varepsilon = +1$; $\Delta g > 0$; positive a_i values).

The distribution of proton hyperfine coupling constants in Met-(S^+), Met-(N^+), and Met-($S^{+}.N$) is expected to be totally different; large values of a_i should only occur for protons on carbon atoms adjacent to the heteroatom(s) bearing the unpaired spin density, that is, for H^3 and H^4 in Met-(S^+), H^1 in Met-(N^+), and H^1 *as well as* H^3 and H^4 in Met-($S^{+}.N$) (compare Scheme 1). As far as Met-(S^+) and Met-(N^+) are concerned, this is again borne out by the CIDNP results[1f] for *S*-methylcysteine and alanine.

The polarization pattern of Met observed at pH 5.8 (Figure 1, bottom trace) therefore clearly points to the sole intermediacy of the sulfur-centered radical cation Met-(S^+). This is not surprising because the amino function is almost completely protonated under these conditions ($pK_{a2} - pH \approx 3.9$) and it therefore cannot function as an electron donor, neither in the quenching process nor in formation of a two-center three-electron bond. In contrast, the polarization pattern at pH 12.2 (about 2.7 units above pK_{a2}, top trace of Figure 1) might be compatible either with the parallel occurrence of Met-(S^+) and Met-(N^+)—that is, with quenching of ^{3}CB by electron transfer from both sulfur and nitrogen—or generation of these polarizations in the radical cation Met-($S^{+}.N$) with the two-center three-electron bond. However, a clear decision between these alternatives is possible by comparison with *S*-methylcysteine, in which the distance between the sulfur and nitrogen termini is shorter by one CH_2 unit. While this shortening cannot have a noticeable influence on the redox potentials of the sulfur center and of the amino function because these groups are still isolated by a saturated C_2 chain, it renders impossible formation of a ($S^{+}.N$) species because this would involve a four-membered ring. The polarization pattern in the photoreaction of CB with *S*-methylcysteine at a pH value well above pK_{a2} of the amino acid unambiguously demonstrates that quenching of ^{3}CB takes place by electron transfer from sulfur only; electron transfer from nitrogen does not participate to any measurable degree.[1f] This finding therefore rules out electron transfer from nitrogen also in the methionine system. The fact that the rate constants for quenching of ^{3}CB by Met at pH 6.8 and 11.5 are practically identical (2.6×10^9 *vs.* 2.7×10^9 M^{-1}s^{-1})[1d]

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provides additional corroboration. The polarization pattern of the top trace in Figure 1 must therefore be due to the radical Met-(S.$^{+}$.N) of methionine with the two-center three-electron bond.

In our high magnetic field (5.9 T) and with the substantial difference Δg of the radical pair involved, there is approximate proportionality between polarization intensity and the hyperfine coupling constant.[8] Furthermore, it is reasonable to assume that the formation of Met-(S.$^{+}$.N) from the sulfur-centered radical cation Met(S$^{+\cdot}$)—which must be the primary methionine-derived radical because in the ground state the interaction between the lone pairs of sulfur and nitrogen is repulsive—is fast on the CIDNP timescale because this reaction is exergonic and the rate-limiting step should be diffusion of the linked sulfur and nitrogen moieties towards one another. Under these conditions the distribution of the unpaired spin density ϱ in the radical cation Met-(S.$^{+}$.N) can be estimated from the limiting ratio of CIDNP intensities P (H^{1}): P (H^{3}) at high pH by using McConnell-type relationships.[12] Taking the dimeric radical cation of tetrahydrothiophene (a (H$^{\alpha}$) = 0.93 mT;[14] this value must be doubled to describe a radical cation in which ϱ is located on a single sulfur atom) and the radical cation of *N*-methylpyrrolidine (average of the splittings the methylene α protons 4.25 mT)[15] as model compounds, one obtains that in the species Met-(S.$^{+}$.N) with the two-center three-electron bond about one third of the unpaired spin density is shifted from sulfur to nitrogen ($\varrho_S : \varrho_N = 0.64 : 0.36$). The calculated ratio ($\varrho_S : \varrho_N = 0.74 : 0.26$)[7] is somewhat higher but, as the authors of reference [7] pointed out, the STO-3G* method used underestimates the spin density at nitrogen.

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[5] Scheme 1 shows the three types of radical cations derived from methionine (sulfur-centered radical cation Met-(S$^{+\cdot}$), nitrogen-centered radical cation Met-(N$^{+\cdot}$), and radical cation Met-(S.$^{+}$.N) with a two-center three-electron bond. The distribution of proton hyperfine coupling constants a in each radical is given schematically below the respective structural formula. Proton H^{1} is attached to the carbon atom bearing the amino group (compare the formula in Figure 1).

[6] For simplicity, protonation equilibria are omitted in Scheme 1 and Figure 1; that is, methionine and its radical cations are displayed in their deprotonated forms, which is present above the respective pK_{a2} value. Because of H/D exchange with the solvent, the nitrogen atom bears no protons but only deuterons in our experiments.

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A Fully Encapsulated Acetylenediide in $Ag_2C_2 \cdot 8\,AgF$**

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Silver acetylide (silver carbide), Ag_2C_2, is highly explosive when dry, and violent decomposition occurs upon mechanical shock and on heating to 120 – 140 °C.[1] Several double salts of silver acetylide with other silver compounds are known,[2–4] but only silver acetylide/silver nitrate (1/6) has been structurally characterized.[5–6]

Up to now the linear structure **I** of silver acetylide (Figure 1) was inferred from its chemical properties, which are consistent with those of a molecular compound but differ greatly from those of the ionic carbides of Group 1 and 2

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