ORGANIC LETTERS

2006
Vol. 8, No. 26
6027−6030

Spin-Polarized Peroxyl Radical Adducts Formed from the Addition of Oxygen to Amino Acid Radicals

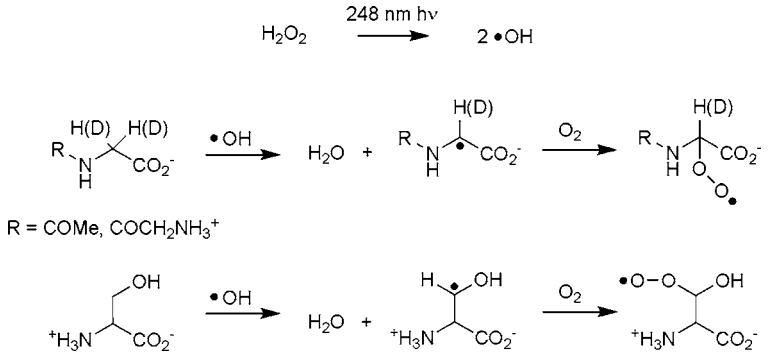
Ryan C. White and Malcolm D. E. Forbes*

Venable and Kenan Laboratories, Department of Chemistry, CB#3290, University of North Carolina, Chapel Hill, North Carolina 27599

mdef@unc.edu

Received October 11, 2006

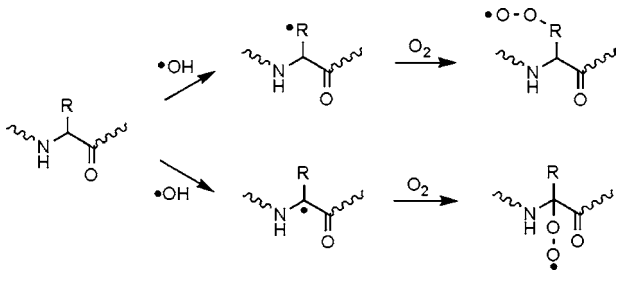
ABSTRACT



Peroxyl radicals formed from the addition of oxygen to carbon radicals of *N*-acetyl glycine, serine, and diglycine are directly observed at room temperature via time-resolved EPR spectroscopy. Isotopic labeling confirms the identity of the *N*-acetyl glycine and diglycine peroxyl analogues. The peroxyl radicals show unusually strong chemically induced electron spin polarization which is discussed in terms of the radical pair mechanism and spin polarization transfer processes.

Certain byproducts of cellular respiration, such as hydrogen peroxide and hydroxyl radicals, are known to oxidatively damage proteins and enzymes.[1] These chemical reactions can lead to a number of unnatural and toxic protein responses such as loss of enzymatic activity and structure,[2] as well as fragmentation and aggregation.[3] It has also been shown that in the presence of oxygen fragmentation of these proteins is preferred, whereas in its absence, aggregation is more prevalent.[4] The mechanism of hydroxyl radical attack on proteins and amino acids in oxygenated environments has been exhaustively reviewed and points to the formation of peroxyl adducts as key intermediates in main chain scission of proteins.[5−7] These adducts are formed by the reaction of O_2 with either the α-carbon radical on the main chain or the carbon or sulfur radicals on side chains (Scheme 1). At ambient temperatures, peroxyl radicals have short chemical lifetimes in aqueous solution[8] and have only been successfully observed in frozen solutions in previous studies. Spin

Scheme 1. Peroxylation of Amino Acid Residues along the Peptide Backbone[a]



[a] R = carbon or sulfur atom.

(1) Davies, M. J. *Biochim. Biophys. Acta* **2005**, *1703*, 93.
(2) Chao, C.; Ma, Y.; Stadtman, E. R. *Proc. Natl. Acad. Sci.* **1997**, *94*, 2969.
(3) Davies, K. J. A.; Lin, S. W.; Pacifici, R. E. *J. Biol. Chem.* **1987**, *262*, 9914.
(4) Davies, K. J. A. *J. Biol. Chem.* **1987**, *262*, 9895.

10.1021/ol062510p CCC: $33.50
Published on Web 11/30/2006

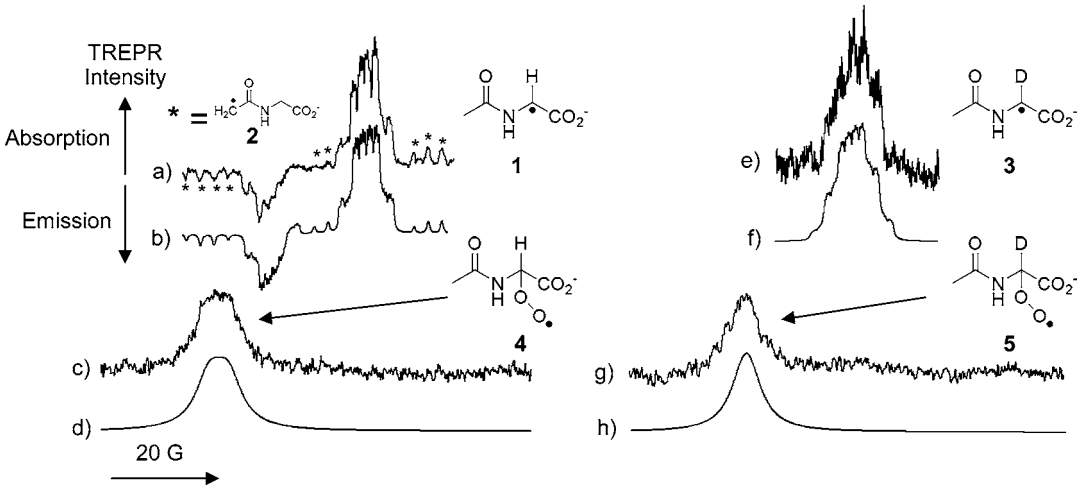


Figure 1. TREPR spectra taken upon 248 nm laser irradiation (1 μs delay) of aqueous solutions (pH 5.5) of 0.8 M H_2O_2 and 0.4 M NAG in (a) deoxygenated and (c) oxygenated solutions with respective simulations (b) and (d) and of 0.1 M NAG-d_2 solution in (e) deoxygenated solution and (g) oxygenated solutions with respective simulations (f) and (h). Magnetic parameters from simulations for **1**: *g*-factor 2.00272; hyperfine coupling constants (hcc) in gauss, H_α 17.5, H_N 1.3, 3H 2.6, N 0.4. For **2**: *g*-factor 2.00240; hcc (G), $2H_\alpha$ 20.9, H_N 2.7, N 2.5. For **3**: *g*-factor 2.00270; hcc (G), D_α 2.8, H_N 1.3, 3H 2.6, N 0.4. For **4**: *g*-factor 2.01300; hcc (G), H 4.0; line width (lw) 5.9 G. For **5**: *g*-factor 2.01380; hcc (G) 0.6; lw 5.9 G.

trapping techniques have been used to trap peroxyl radical adducts arising from radical reactions of amino acids and proteins;[9] to the best of our knowledge, these adducts have never been directly observed by EPR techniques in real time, in 100% aqueous solutions, at room temperature.

Previously, we have used time-resolved EPR (TREPR)[10] to observe radicals formed from the primary oxidation steps of biologically relevant amino acids[11] and peptides,[12] as well as electron-transfer processes in peptides.[13] Here we report the direct observation of peroxyl radicals generated upon UV photolysis of hydrogen peroxide in oxygenated solutions via TREPR spectroscopy from the substrates *N*-acetyl glycine (NAG), diglycine, and serine. Unlike steady-state spectra which rely on Boltzmann populations of spin levels, the phase and intensity of TREPR spectral transitions are affected by chemically induced dynamic electron polarization (CIDEP) mechanisms. The observed polarization patterns can yield mechanistic information about the radical pairs. For the spectra observed here, the peroxyl radical signal obtains polarization strength from the originally created substrate radicals, making their observation facile at room temperature in real time.

Figure 1a is the spectrum obtained upon photolysis of H_2O_2 in the presence of NAG and is assigned to the α-carbon radical **1**. Parameters obtained from computer simulation of this spectrum (Figure 1b) agree with those obtained by Neta and Fessenden[14] and Hawkins and Davies.[15] A small signal corresponding to radical **2** is also observed. Both of these radicals are formed by H abstraction and are stabilized by adjacent carbonyl groups.

The low-field lines in Figure 1a (and for all spectra presented) appear in emission (E), and the high-field lines are absorptive (A). This pattern (which we call E/A) is attributed to the radical pair mechanism (RPM) of CIDEP. The original radical pair consists of two hydroxyl radicals generated from the excited singlet state of H_2O_2, which would, according to Kaptein's rules for CIDEP,[16] give geminate polarization that is A/E. However, hydroxyl radicals react at near diffusion controlled rates and have fast electron spin relaxation; therefore, this geminate pair CIDEP would be difficult to observe. The E/A pattern observed in the spectra in Figure 1 must be generated in random or so-called F-pairs. These F-pairs consist of substrate radicals, peroxyl radicals, and perhaps residual •OH radicals. It should be noted that there is more A than E overall in the spectra. This could be attributed to a spin-selective reaction, originally proposed by Paul and Fischer,[17] between the substrate radical and the remaining H_2O_2. The reaction depletes certain spin states of the substrate radicals, leading to an overall E/A* pattern. The E/A* intensities may also be attributed to a superposition of different observed F-pairs. This latter explanation seems more likely and will be elaborated upon further in a later publication.

Figure 1c shows spectra taken upon irradiation of aqueous H_2O_2/NAG solutions that have been oxygen saturated. A

(5) Garrison, W. M. *Chem. Rev.* **1987**, *87*, 381.
(6) Hawkins, C. L.; Davies, M. J. *Biochim. Biophys. Acta* **2001**, *1504*, 196.
(7) Headlam, H. A.; Davies, M. J. *Free Radical Biol. Med.* **2002**, *32*, 1171.
(8) Neta, P.; Huie, R. E.; Ross, A. B. *J. Phys. Chem. Ref. Data* **1990**, *19*, 413.
(9) Davies, M. J.; Fu, S.; Dean, R. T. *Biochem. J.* **1995**, *305*, 643.
(10) Forbes, M. D. E. *Photochem. Photobiol.* **1997**, *65*, 73.
(11) Clancy, C. M. R. *Photochem. Photobiol.* **1999**, *69*, 16.
(12) White, R. C.; Tarasov, V. F.; Forbes, M. D. E. *Langmuir* **2005**, *21*, 2721.
(13) Burns, C. S.; Rochelle, L.; Forbes, M. D. E. *Org. Lett.* **2001**, *3*, 2197.
(14) Neta, P.; Fessenden, R. W. *J. Phys. Chem.* **1971**, *75*, 738.
(15) Hawkins, C. L. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2617.
(16) Kaptein, R.; Oosterhoff, L. J. *Chem. Phys. Lett.* **1969**, *4*, 195.
(17) Paul, H.; Fischer, H. *Z. Naturforsch., Teil A* **1970**, *25*, 443.

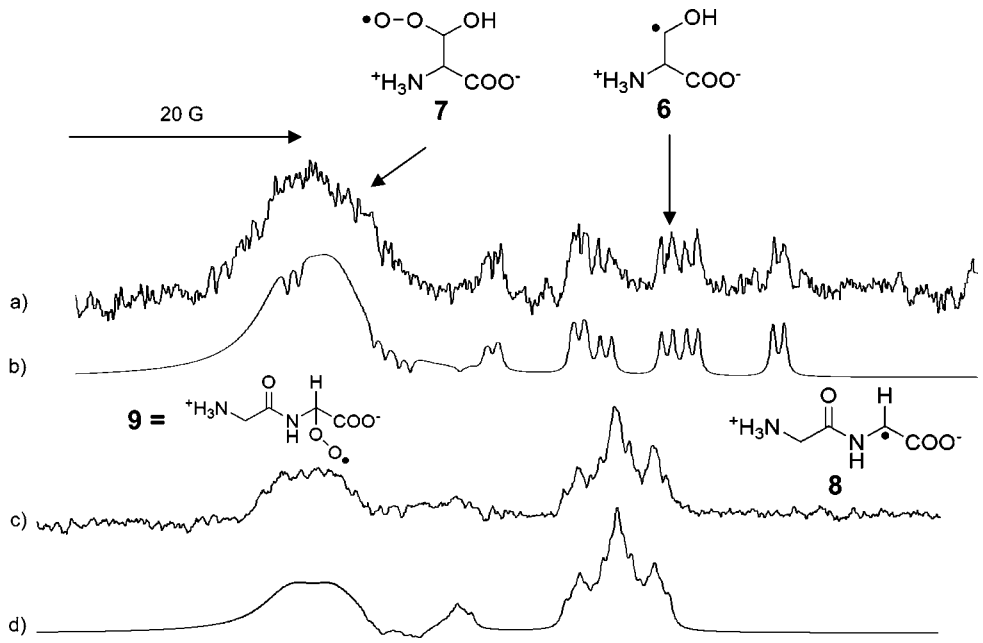


Figure 2. TREPR spectra taken upon 248 nm laser irradiation of oxygenated aqueous solutions (pH 5.5) of 0.8 M H_2O_2 and 0.4 M (a) serine and (c) diglycine solutions. Simulations are shown in (b) and (d), respectively. Magnetic parameters from simulations for **6**: *g*-factor 2.00290; hcc (G), H_a 17.7, H_β 9.8, N 7.6, H_O 1.0. For **7**: *g*-factor 2.01462; hcc (G), H 4.0; lw 4.7 G. For **8**: *g*-factor 2.00292; hcc (G), H_α 17.4, H_N 1.2, 2H 3.3, 0.7 N. For **9**: *g*-factor 2.01380; hcc (G), H 4.0; lw 4.7 G.

broad signal appears at low field, and the transitions due to radical **1** have disappeared completely. The broad signal has a *g*-factor of 2.0130, consistent with known literature values for peroxyl radicals.[18] To confirm that we are observing the peroxyl-NAG radical adduct and not •OOH or another transient species, the same experiment was run with isotopically substituted *N*-acetyl glycine-2,2-d_2 (NAG-d_2) in both deoxygenated (Figure 1e) and oxygenated (Figure 1g) conditions. Under N_2, radical **3**, the deuterated analogue of **1**, is formed. The line width of the peroxyl radical in Figure 1f is noticeably smaller than that in the protonated case shown in Figure 1c. This narrowing of the line width occurs because the largest hyperfine coupling constant has been changed from H to D by a factor of 6.5. The broad transitions observed in Figures 1c and 1g are therefore assigned to peroxyl radical adducts **4** and **5**, respectively. The line width of 5.9 G, as well as hyperfine interactions of 4.0 G (**4**) and 0.6 G (**5**), were used in the simulations shown in Figures 1d and 1h. These hyperfine values are comparable with those found previously for peroxyl adducts observed in polar nonaqueous solvents via steady-state techniques.[18,19]

The absorptive CIDEP observed in peroxyl radicals **4** and **5** can be explained by polarization transfer. Because the polarization of the TREPR signal is inherited directly from the carbon parent radical and the net polarization of the carbon radicals are absorptive (E plus enhanced A = overall A or E/A*), the peroxyl radicals will show net A polarization.

To test the generality of this chemistry, other amino acid and peptide analogues were analyzed. When glycine was used as a substrate, no signal was observed in deoxygenated or oxygenated solutions, though α-carbon radicals from this substrate have been observed via steady-state EPR at ambient temperatures.[14,15] The ^{17}O peroxyl adduct of alanine has been observed and characterized via steady-state conditions in frozen aqueous solution by Sevilla et al.[20] When we ran the TREPR experiment with H_2O_2 and alanine, we observed the C(3) primary radical under deoxygenated solution. When the same experiment was run in oxygen-saturated solutions, the peroxyl radical signal was not directly observed. However, the intensity of the signal from the carbon parent radical was reduced in the presence of oxygen. It is possible that for these cases the reaction with O_2 is too slow to allow for polarization transfer or that recombination of the F-pairs is fast and no spin-selective depletion is observed.

TREPR spectra and simulations performed with the serine and glycyl−glycine are shown in Figure 2. The goal of the serine experiment was to observe the peroxyl adduct on a radical center neighboring hydroxyl group. The spectrum shown in Figure 2a shows two different radicals, the serine C(3) carbon parent radical **6** and the peroxyl radical adduct **7**. Radical **6** has been observed by Behrens and Koltzenburg in aqueous solution,[21] and the magnetic parameters used in the simulation in Figure 2b are similar. The intensity of the transition due to **7** is greatest at 1.5 μs. Because both radicals are seen in solution at the same time, a delay means that [**6**]

(18) Kitaguchi, H.; Ohkubo, K.; Ogo, S.; Fukuzumi, S. *J. Am. Chem. Soc.* **2005**, *127*, 6605.
(19) Miyamoto, S.; Martinez, G. R.; Rettori, D.; Augusto, O.; Medeiros, M. H. G.; Di Mascio, P. *Proc. Natl. Acad. Sci. U.S.A* **2005**, *103*, 293.
(20) Sevilla, M. D.; Becker, D.; Yan, M. *Int. J. Radiat. Biol.* **1990**, *57*, 65.
(21) Behrens, G.; Koltzenburg, G. *Z. Naturforsch.* **1985**, *40c*, 785.

is greater than $[O_2]$ and oxygen is the limiting reactant to form **7**.

Diglycine was also tested under these experimental conditions to examine the effect of a protonated ammonium N-terminus on the formation of the peroxyl adduct. Figures 2c and 2d show the spectrum and computer simulation. The spectrum is similar to the serine case in which both the α-carbon (**8**) and the peroxyl adduct (**9**) radicals are observed. Hawkins and Davies reported that the majority (90%) of •OH attack occurs on the α-carbon neighboring the carboxylate group.[14] This is indeed the only observed radical. It can be assumed that the observed peroxyl radical peak is attributed to the peroxyl radical adduct at this site.

These reactions show significant spin polarization of peroxyl radicals in room-temperature solutions and provide a new avenue for the study of an important class of reactive intermediates. Because peroxyl radicals have *g*-factors that are quite different from carbon-centered radicals, they are easy to identify and the extension of this chemistry to short peptides and proteins under physiological conditions should be possible. It is also important to note that this method of generating peroxyl radicals avoids the presence or generation of singlet oxygen, which often complicates such photochemistry. In a forthcoming full paper, we will show further that rate information can also be extracted from this experiment by monitoring the kinetics of the transitions from each species.

Acknowledgment. The authors would like to thank the National Science Foundation (Grant No. CHE-0518300) for support of this project.

OL062510P