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ARTICLES

Time Resolved FT EPR Identification of (E) and (Z) Conformational Isomers of Glycyl Radicals Formed upon Photoinduced Oxidation of Glycine Esters in Aqueous Solutions

Peter Tarábek,[†] Marija Bonifačić,[‡] Sergei Naumov,[§] and Dieter Beckert*,[†]

Interdisciplinary Research Group "Time-Resolved Spectroscopy", Faculty of Chemistry and Mineralogy, University of Leipzig, Permoserstrasse 15, Leipzig, D-04318 Germany, Department of Physical Chemistry, Ruđer Bošković Institute, Bijenička c. 54, HR-10 000 Zagreb, Croatia, and Leibniz-Institute of Surface Modification, Leipzig, Permoserstrasse 15, Leipzig, D-04318 Germany

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Using time-resolved Fourier transform electron paramagnetic resonance (FT EPR) spectroscopy, the photooxidation of glycine methyl and ethyl esters by spin-polarized triplets of anthraquinone-2,6-disulfonate was studied in aqueous solution in the pH range 9−10. Quinone radical anions showing strong emissive spin-polarization (CIDEP) were formed, indicating fast electron transfer from the glycine esters to the spin-polarized quinone triplet as the primary reaction. The lifetime of the simultaneously formed glycine ester aminium radical cations was too short for detection by FT EPR in the nanosecond time-scale. Fast deprotonation of aminium radicals from nitrogen and α-carbon sites led to the generation of two successor radicals, the aminyl radical $^{\bullet}NH-CH_2-CO_2R$ and the glycyl radical $NH_2-^{\bullet}CH-CO_2R$ (R = Me or Et) in the ratio of 1:9, respectively. Glycyl radicals were found to be present in two isomeric forms (Z and E), as unambiguously identified from their FT EPR spectra. The assignment to the two isomers was supported by quantum chemical DFT calculations. The calculated *hfc* constants for aminyl radicals and for (E)/(Z) glycyl radicals are in accordance with experimental values. The concentration ratio of (Z) and (E) isomers was determined to be 7:3. No interconversion was observed within 1 μs at room temperature. Results are discussed in the light of their relevance to the oxidation mechanism and, in particular, to the enantioselective synthesis of aliphatic amino acids and their derivatives.

Introduction

Amines are among the most investigated compounds used as characteristic electron donors in oxidation processes initiated by a number of different methods, particularly in the photochemically induced electron-transfer reactions.[1−4] The triplet quenching rate constants by amines are often high, up to the diffusion-limited values.[5] As shown in a general reaction 1, the primary step of such an electron-transfer quenching process is the formation of an aminium radical cation and a sensitizer radical anion in the solvent cage:

$$A_T + NHR\text{-}CHR_2 \rightarrow \{A^{\bullet-} + {}^{+\bullet}NHR\text{-}CHR_2\} \qquad (1)$$

Aminium radicals are highly reactive species usually undergoing fast chemical changes.[3] The most known process in polar solvents is the prompt deprotonation either from the α-C−H position or from the thermodynamically more acidic N−H group in the case of primary or secondary amines,[4] yielding reducing α-amino-C-centered radicals (reaction 2) and oxidizing aminyl

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* To whom correspondence should be addressed. E-mail: beckert@mpgag.uni-leipzig.de.
[†] University of Leipzig.
[‡] Ruđer Bošković Institute.
[§] Leibniz-Institute of Surface Modification.

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radicals (reaction 3), respectively

$$^{+\bullet}NHR{-}CHR_2 \rightarrow NHR{-}{}^{\bullet}CR_2 + H^+ \quad (2)$$

$$^{+\bullet}NHR{-}CHR_2 \rightarrow {}^{\bullet}NR{-}CHR_2 + H^+ \quad (3)$$

Aminium radicals derived from amines bearing different functional groups can undergo mechanistically similar fragmentation reactions.[2,4] One of the most interesting in this series is the decarboxylation of amino acid aminium radicals, shown for glycine by reaction 4. This leads to the irreversible degradation of α-amino acids, compounds of fundamental importance in biochemical processes in nature and also in technology:

$$^{+\bullet}NH_2{-}CH_2{-}CO_2^- \rightarrow {}^{\bullet}CH_2{-}NH_2 + CO_2 \quad (4)$$

For aminium radicals derived from aliphatic α-amino acid anions in basic aqueous solutions, this is an extremely fast process occurring with a rate constant of about 10^{11} s^{-1}.[6] It successfully competes with the deprotonation processes, as the corresponding α-amino-α-carboxyalkyl radicals (glycyl anion for the amino acid glycine)[7] and aminyl radical anions[6] were formed only as minor products by using anthraquinone or benzophenone triplets as oxidation agencies.[8−10]

Following our interest to further explore the oxidation mechanism of aliphatic amino acids in aqueous media, in this study, we employed glycine alkyl esters as electron donors for quenching of anthraquinone-2,6-disulfonate triplets. We are not aware of any study on the mechanism of the one-electron oxidation processes of this class of amino acid derivatives. Esterification should, however, dramatically change the fate of primarily formed aminium radicals. It prevents their decarboxylation allowing deprotonation to be the only pathway for their stabilization. By using the time-resolved FT EPR detection method, the resulting radicals could be directly identified giving information on the side of deprotonation (amino versus CH_2 group). Assuming that the bond energies and acidities of α−C−H and N−H groups in esters are essentially the same as in the parent glycine molecule, the results are expected to be relevant for aliphatic amino acids in general. Further on, the usage of esters as electron donors turned out to be a convenient and efficient method to produce uncharged aminyl and glycyl radicals, thus allowing investigations of their properties in almost neutral aqueous solutions.

Aminyl radicals have been only recently identified as intermediates in the aliphatic amino acids oxidation processes,[6,9−13] including determination of some of their chemical and physicochemical properties. Data of the paramagnetic properties of the aminyl type of radicals are scarce in the literature. These radicals are relatively stable species[6,11,14] not prone to undergo fast intramolecular transformations. For example, 1,2-hydrogen shift, a conversion of aminyl into thermodynamically more stable glycyl radicals as shown by reaction 5 for the glycine aminyl radical anion, has, according to DFT calculations, a rather high transition state activation energy.[11] It is interesting to note that another possible aminyl/α-C-centered radical transformation, the intermolecular hydrogen atom abstraction, proceeds only moderately fast, $k = 3 \times 10^4$ M^{-1} s^{-1} for glycine, reaction 6[9]

$$^{\bullet}NH{-}CH_2{-}CO_2^- \rightarrow NH_2{-}{}^{\bullet}CH{-}CO_2^- \quad (5)$$

$$^{\bullet}NH{-}CH_2{-}CO_2^- + NH_2{-}CH_2{-}CO_2^- \rightarrow NH_2{-}CH_2{-}CO_2^- + NH_2{-}{}^{\bullet}CH{-}CO_2^- \quad (6)$$

The α-C-centered glycyl radicals, $NH_2{-}{}^{\bullet}CH{-}CO_2^-$ and $NH_2{-}{}^{\bullet}CH{-}CO_2H$, are particularly stable species, with additional stabilization arising from the capto-dative effects of the neighboring functional groups. The same can be anticipated for the $NH_2{-}{}^{\bullet}CH{-}CO_2R$ form studied here. They can be produced also by a direct H-atom abstraction from the α-C−H position by different radicals in reactions similar to 6. Interestingly, the reactions rate constants are generally low,[15] being well below diffusion limits even for the reaction of $^{\bullet}OH$ with Gly^-.[10] Due to a relatively low bond energy and a high resonant stabilization of the resulting glycyl type of radicals, the α-C−H group of glycine residues is believed to be a favored site of attack by radicals in amino acid derivatives and small peptides.[16]

Incidentally and to our surprise, the EPR spectra observed following photooxidation of glycine esters in the magnetic field region characteristic for glycyl radicals were very complex and seemed initially difficult to interpret. However, just this part of the study has emerged as the most interesting. These neutral radicals, $NH_2{-}{}^{\bullet}CH{-}CO_2Me/Et$, have been identified as the major intermediates. Moreover, they were present in two distinct isomeric forms at room temperature, unambiguously identified as (E) and (Z) conformers. The phenomenon that radicals show isomerism on trigonal carbon atom has been often discussed in the literature, particularly to explain results of the enantioselective syntheses of a number of compounds including aliphatic amino acids and their derivatives, as well as peptides (for reviews see refs 17 and 18). Experimental evidences, obtained by means of the EPR technique, exist however only for (E)/(Z) isomers of methallyl radicals[19] and for several C-centered radicals with oxygen and sulfur-containing substituents.[20] Very impressive indirect evidences for the glycyl type of radicals to adopt distinct conformations that stereocontrol the syntheses of amino acids comes from the work of Hamon and co-workers.[21;22]

In this publication, we present for the first time direct EPR experimental evidences for glycine alkyl esters derived glycyl radicals to exist in two (E) and (Z) forms, the latter being favored. Upon photooxidation of glycine esters corresponding aminyl radicals were also identified as the only other intermediate product. Presented are also quantum chemical DFT calculations to assign *hfc* parameters to the geometry of the investigated radicals. More experimental data involving quenching rate constants, and some other kinetical and mechanistic analysis of the investigated systems will be published separately in a forthcoming publication.

Experimental Section

The 308 nm XeCl line of an excimer laser (Lambda Physik, LPX 105 ESC; energy 10−30 mJ per pulse; pulse width 16 ns) was used for photoexcitation. The FT EPR equipment has been described previously.[23−25] The power of the microwave pulse used in the experiments was 20 W with a pulse length for the $\pi/2$ pulse of 48 ns. The excitation width in the spectra was thus about $\Delta B = \pm 0.5$ mT. The cavity was the Bruker splitring module ER 4118X-MS-5W. The receiver dead time was on the order of 80−100 ns. All experimental FID data were extrapolated using the linear prediction singular value decomposition method (LPSVD).[24;26]

Anthraquinone-2,6-disulfonic acid disodium salt (2,6-AQDS), glycine methyl ester hydrochloride (99%), GlyOMe, and glycine ethyl ester hydrochloride (99%), GlyOEt, were purchased from Aldrich and were used without further purification. Water was taken from a milli-Q plus ultrapure water system (Millipore). Deuterium oxide (99.8%) from Deuchem was used for measurements with D_2O as solvent. The solution flowed through the

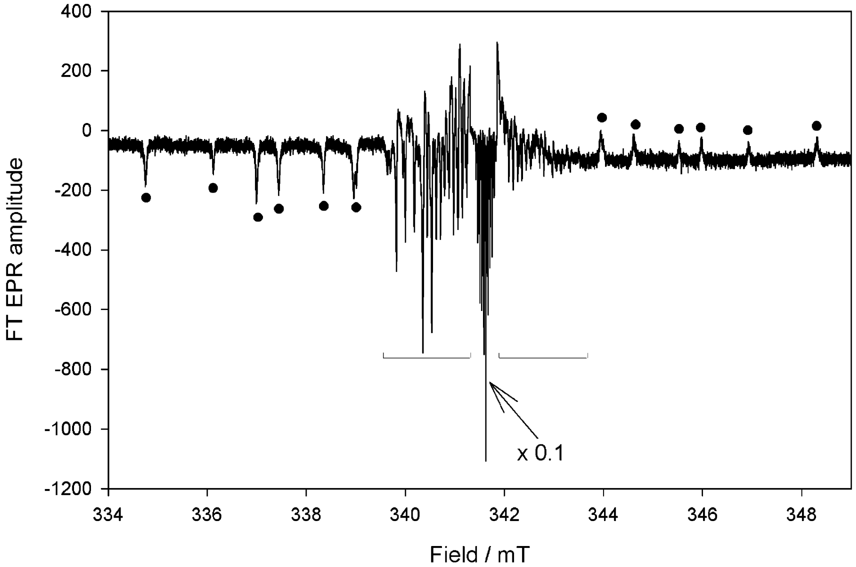

Figure 1. FT EPR spectrum of the radical anion 2,6-AQDS$^{•-}$* and the successor radicals of the radical cation GlyOMe$^{•+}$* at a delay time of 96 ns after the laser pulse in aqueous solution. Sample: 1 mM 2,6-AQDS, 50 mM GlyOMe, pH 9.3, 5 °C.

EPR tube (optical path length about 1.0 mm) at a rate of about 2−3 mL/min to avoid depletion of the sensitizer and/or enrichment of reaction products. The double-sided glass tube system before the resonator allows the temperature to be varied (for aqueous systems between 5 and 80 °C). The measurements were carried out at the pH range between 8.5 and 10 where investigated esters exist in their deprotonated form ($pK_a = 7.7-7.8$). KOH (Aldrich) was used to adjust the pH. To remove oxygen, the samples were bubbled with argon (99.99%) before (about 20 min) and during the whole experiment. All measurements were carried out at room temperature unless otherwise indicated.

Results and Discussion

The complete FT EPR spectrum obtained in an aqueous solution containing 1 mM 2,6-AQDS and 50 mM GlyOMe at a delay time of 96 ns between the laser and the microwave detection pulses is shown in Figure 1. With GlyOEt as electron donor, a similar spectrum was measured (not shown here). The spectrum in Figure 1 was composed from several measurements with the field offsets $\Delta B_0 = (0, \pm0.5, \pm1, ..., \pm6.5)$ mT. The central line group is due to the radical anion 2,6-AQDS$^{•-}$ which shows a strong emissive EPR intensity (multiplied by 0.1 for better presentation). This emissive spectrum of 2,6-AQDS$^{•-}$ indicates the electron transfer from the amino group of glycine esters to the 2,6-AQDS triplet as the main primary quenching reaction in the photooxidation of GlyOMe (GlyOEt):

$$\text{2,6-AQDS}_T^* + \text{GlyOMe} \rightarrow \text{2,6-AQDS}^{•-*} + \text{GlyOMe}^{•+*} \qquad (7)$$

where spin-polarized species are denoted by * and the 2,6-AQDS triplet is spin-polarized by the CIDEP triplet mechanism.[27,28] The EPR spectrum of the radical anion 2,6-AQDS$^{•-}$ is described in previous papers[23,29] and will not be further discussed here.

The radical cations GlyOMe$^{•+}$* and GlyOEt$^{•+}$* formed in the primary electron-transfer process (7) could be with little doubts ascribed to the aminium radicals $^{+•}NH_2-CH_2-CO_2R$. They were, however, not detectable with our FT EPR set up, most probably due to their very short lifetimes. Therefore, the FT EPR spectra (besides the lines of the 2,6-AQDS$^{•-}$ radical anion) have to be assigned to their successor radicals. The analysis of the structure of the line groups in Figure 1 that belong to the glycine ester radicals results in three different subspectra, the first designated by (●), the second and the third by (⎵).

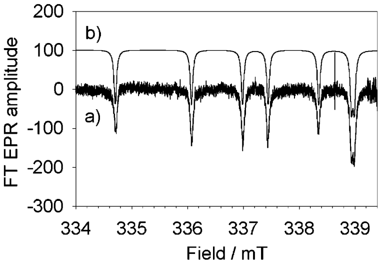

Figure 2. Low field part of the FT EPR spectrum of the aminyl radical $^•NH-CH_2-CO_2Me$ in H_2O at a delay time of 96 ns (a) in comparison with the simulated spectrum (b). Simulation parameters are given in Table 1. Sample: 1 mM 2,6-AQDS, 100 mM GlyOMe, pH 9.1, room temperature.

It should be mentioned at this point that the spectrum shown in Figure 1 has been taken at conditions ensuring clear demonstration of all produced EPR lines. The polarization intensities of individual radicals do not necessarily represent their primary yields. This is because at the time of spectrum recording some of the glycine ester derived radicals have already been partially converted into 2,6-AQDS$^{•-}$ in their reaction with ground state 2,6-AQDS molecules.

As it will be shown later (see part 2 of this section), the spectra also contain lines of an additional radical, a product characteristic for one-electron reduction reactions of glycine esters. The radical was formed in a minor yield in a hard to avoid side reaction. This subspectrum is designated by (§) in Figures 4 and 5.

1. Structure of the GlyOR$^{•+}$ Radical Successors and Mechanism of Their Formation. *1.1. Aminyl Radicals.* Figures 2 and 3 show the low field parts of the subspectrum (●) for GlyOMe measured in H_2O and D_2O, respectively. This group of lines can be ascribed to the aminyl radicals $^•NH-CH_2-CO_2Me$ and $^•ND-CH_2-CO_2Me$. The measured spectra are compared with the simulated ones (spectra b) in Figures 2 and 3. The *hfc* parameters used for simulations are collected in Table 1 which also contains corresponding data obtained for GlyOEt

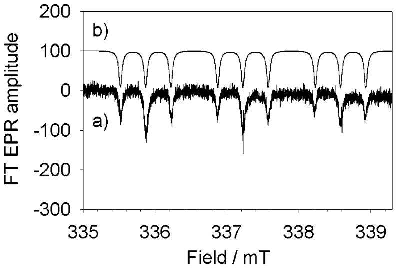

Figure 3. Low field part of the FT EPR spectrum of the radical $^{\bullet}$ND$-$CH$_2-$CO$_2$Me in D$_2$O at a delay time of 96 ns (a) in comparison with the simulated spectrum (b). Simulation parameters are given in Table 1. Sample: 1 mM 2,6-AQDS, 100 mM GlyOMe, pD 9.6, room temperature.

TABLE 1: EPR Parameters of Glycine Ester Radicals Determined by Simulation of the FT EPR Spectra[a]

radical	a_N	$a_{NH(D)}$	a_{CH^1}	a_{CH^2}	$a_{COMe(Et)}$	g factor
$^{\bullet}$NH$-$CH$_2-$CO$_2$Me	1.368	2.279	4.230	4.281		2.00433
$^{\bullet}$ND-CH$_2-$CO$_2$Me	1.356	0.351	4.230	4.281		2.00440
$^{\bullet}$NH$-$CH$_2-$CO$_2$Et	1.364	2.280	4.226	4.277		2.00432
$^{\bullet}$ND-CH$_2-$CO$_2$Et	1.356	0.351	4.245	4.300		2.00433
NH$_2-^{\bullet}$CH$-$CO$_2$Me(Z)	0.620	(2) 0.515	1.208		(3) 0.175	2.00354
ND$_2-^{\bullet}$CH$-$CO$_2$Me(Z)	0.605	(2) 0.084	1.220		(3) 0.177	2.00356
NH$_2-^{\bullet}$CH$-$CO$_2$Me(E)	0.625	(2) 0.527	1.181		(3) 0.127	2.00364
ND$_2-^{\bullet}$CH$-$CO$_2$Me(E)	0.614	(2) 0.084	1.189		(3) 0.129	2.00365
NH$_2-^{\bullet}$CH$-$CO$_2$Et(Z)	0.618	(2) 0.515	1.208		(2) 0.169	2.00353
ND$_2-^{\bullet}$CH$-$CO$_2$Et(Z)	0.607	(2) 0.084	1.210		(2) 0.177	2.00356
NH$_2-^{\bullet}$CH$-$CO$_2$Et(E)	0.622	(2) 0.528	1.183		(2) 0.124	2.00363
ND$_2-^{\bullet}$CH$-$CO$_2$Et(E)	0.608	(2) 0.084	1.190		(2) 0.126	2.00364
$^{\bullet}$CH$_2-$COOMe			2.131	2.131	(3) 0.157	2.00339
$^{\bullet}$CH$_2-$COOEt			2.122	2.122	(2) 0.161	2.00339

[a] The *hfc* constants are given in mT. In parenthesis are indicated the number of equivalent nuclei.

derived radicals. Considering the differences in spectra measured in H$_2$O and D$_2$O, the assignment of the *hfc* parameters to the different coupling nuclei is unambiguous. The *hfc* parameters obtained are similar to the data determined by Fessenden et al. for the aminyl radical $^{\bullet}$NH$-$C(CH$_3$)$_2-$CO$_2^-$ derived from α-methylalanine[13] in aqueous solution and by Ingold et al. for *N*-alkoxy-aminyl radicals[30] in isopentane or toluene. Our results are also in agreement with the *hfc* constants of aminyl radicals derived from γ-irradiated glycine in solid phase at low temperatures.[31-33]

1.2. Glycyl Radicals. The FT EPR subspectrum in the field range of 339.5$-$344 mT (labeled by ⊔ in Figure 1) is composed of two spectra (cf. Figure 4 for GlyOMe and Figure 5 for GlyOEt showing measured and simulated spectra). Each of these spectra can be simulated with one nitrogen nucleus carrying two equivalent exchangeable protons, one additional proton, and three (for GlyOMe) or two (for GlyOEt) protons with the smallest *hfc* constants**.** The *hfc* constants of the two spectra are similar but not identical for all nuclei (cf. Table 1). By comparison of the *hfc* constants for radicals derived from GlyOMe and GlyOEt in H$_2$O and D$_2$O (for D$_2$O spectra not shown), the assignment of values to the differently *hfc*-active but identical nuclei is unambiguous as shown in Table 1. Accordingly, we interpret these two spectra as the respective (Z)- and (E)-isomeric structures of the glycyl radicals NH$_2-^{\bullet}$CH$-$CO$_2$Me (Figure 4) and NH$_2-^{\bullet}$CH$-$CO$_2$Et (Figure 5).

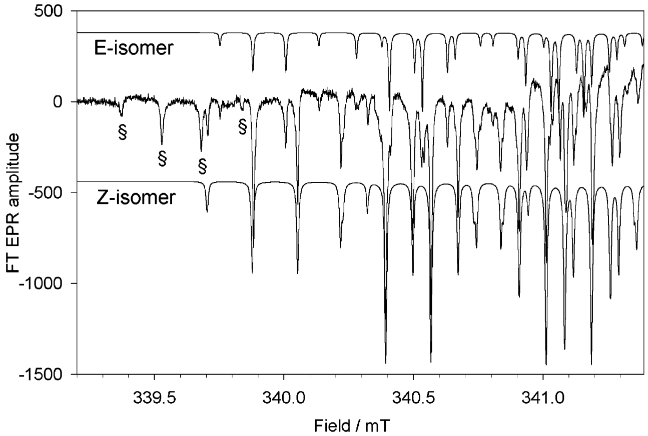

Figure 4. Low field part of the FT EPR spectrum of the glycyl radicals NH$_2-^{\bullet}$CH$-$CO$_2$Me in H$_2$O at a delay time of 96 ns in comparison with the simulated spectra for (E) and (Z) isomers. Simulation parameters are given in Table 1. For lines indicated by § see text. Sample: 0.1 mM 2,6-AQDS, 100 mM GlyOMe, H$_2$O, pH 8.9, room temperature.

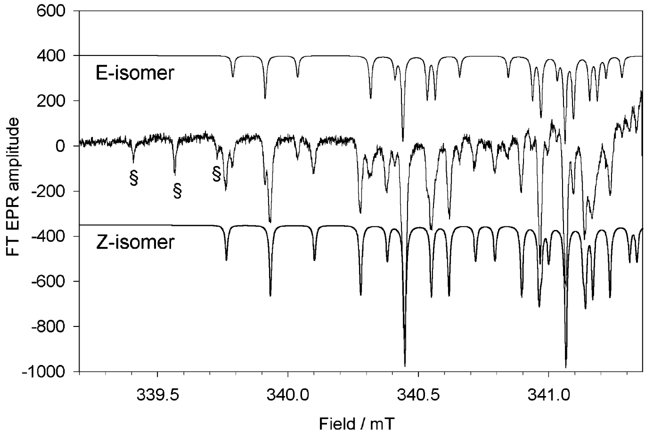

Figure 5. Low field part of the FT EPR spectrum of the glycyl radicals NH$_2-^{\bullet}$CH$-$CO$_2$Et in H$_2$O at a delay time of 96 ns in comparison with the simulated spectra for (E) and (Z) isomers. Simulation parameters are given in Table 1. For lines indicated by § see text. Sample: 0.1 mM 2,6-AQDS, 100 mM GlyOEt, pH 9.3, room temperature.

The EPR spectrum of the glycyl radical, NH$_2-^{\bullet}$CH$-$CO$_2$H, in aqueous solutions was measured by Neta and Fessenden[34] and Paul and Fischer.[35] Their *hfc* constants are similar to our data without any indication for the existence of isomers, which can be explained by a fast exchange process on the time scale of the steady-state EPR detection used in these studies. There is, however, a small difference in the *g* values obtained which may well be due to the different standards applied for their determination (we determine the *g* values by comparison with the known *g* value of the radical anion 2,6-AQDS$^{\bullet -}$ of 2.00412[23]). Also the *hfc* parameters of the glycyl radical anion,[7] NH$_2-^{\bullet}$CH$-$CO$_2^-$, differ slightly from the results obtained in this study for the neutral radicals NH$_2-^{\bullet}$CH$-$CO$_2$Me and NH$_2-^{\bullet}$CH$-$CO$_2$Et.

The results in Table 1 indicate a small decrease of the nitrogen *hfc* coupling by deuteration of the amino group. These nitrogen *hfc* constants are higher in the E isomers in comparison to the Z isomers. The *hfc* constants of the α-C-hydrogen, $>^{\bullet}$C$-$H, and of the hydrogens from the ester groups decrease slightly in the E isomers. To assign these *hfc* parameters to the radical geometry, quantum chemical DFT calculations were carried out, and their results are presented and discussed in detail in part 3.

For both esters, the concentration ratio of the two isomers [Z]:[E] $\sim$ 7:3 at room temperature was estimated by comparison of signals intensities at 96 ns after the pulse. This ratio was not changed up to the time of about 1 μs setting the limit for the interconversion process to be $<10^6$ s^{-1}. Measurements at longer times, allowing the rate of interconversion to be defined more precisely, were not possible under the conditions used, because of the fast glycyl radicals decay in their reaction with ground state 2,6-AQDS molecules present in solutions (concentrations of at least 0.1 mM had to be used). That the isomerization of radicals having conformational isomers could in principle be a relatively slow process has been already shown for the methallyl radicals, $k < 10^2\ \mathrm{s}^{-1}$ in net *cis*-butene-2 at 0 to −130 °C[19] and for the methoxy(methylcarbonyl)methyl radical isomers (no interconversion even at +34 °C).[20]

1.3. Mechanistic Aspects. Under experimental conditions given above, both aminyl and glycyl radicals were fully formed within the first 96 ns after the laser pulse and with the same kinetics as 2,6-AQDS$^{\bullet-}$ radical anions. This suggests very fast deprotonation processes from N−H and α-C−H positions of the aminium radical precursor as their respective formation mechanism. At the same time, such fast formation excludes in principle possible conversion of aminyl radicals, by reactions similar to (5) and (6), to be the source of the glycyl radicals in this measurements. Aminyl radicals and glycyl radicals (both isomeric forms) were formed in the relative concentration ratio of about 1:9. Thus, aminium radical cations derived from glycine methyl and ethyl ester deprotonate preferentially from α-C−H position (reaction 2). Whether α-C−H deprotonation might occur also in the case of aminium radicals $^{+\bullet}\mathrm{NHR{-}CHR{-}CO_2}^-$ (R = H or alkyl group) formed from aliphatic amino acids[14] where the ultrafast decarboxylation prevents such determinations[6] can still not be answered with certainty, but the above results indicate at least that the process might be possible. Finally, it could be concluded that esterification not only enables to conduct oxidation of amino acids under less basic conditions (lowers amino group pK_a values) and protects them from degradation via decarboxylation but in addition makes able fast production of glycyl type of radicals in preferential isomeric forms, most probably responsible for asymmetric induction in chiral products syntheses.[17,18]

2. Side Reactions. The remaining lines labeled by § in Figures 4 and 5 belong to the alkyl radicals $^\bullet\mathrm{CH_2CO_2Me}$ and $^\bullet\mathrm{CH_2CO_2Et}$, respectively, where the central quartet (triplet) is hidden in the strong 2,6-AQDS$^{\bullet-}$ signal. The *hfc* constants determined by simulation are in agreement with the data obtained by Behrens[36] for the same radicals. In our systems, they were formed as minor products only.

Such radicals have been proposed as products of the reductive deamination of glycine ester cations by hydrated electrons, reaction 8:[37]

$$e_{aq}^{\bullet-} + {}^+\mathrm{H_3NCH_2CO_2Me} \rightarrow \mathrm{NH_3} + {}^\bullet\mathrm{CH_2CO_2Me} \qquad (8)$$

In the photoreduction of the 2,6-AQDS triplet by different electron donors (DH), the EPR spectrum of the hydrated electron was observed.[38−40] Its production is due to the two-photon ionization of AQDS via the reaction sequence

$$\mathrm{AQDS} \xrightarrow{h\nu} \mathrm{AQDS_{S1}} \xrightarrow{\mathrm{ISC}} \mathrm{AQDS_{T1}}^* \xrightarrow{+\,\mathrm{DH}} \{\,\mathrm{AQDS}^{\bullet-*}...\mathrm{DH}^{\bullet+*}\} \qquad (9)$$

$$\mathrm{AQDS}^{\bullet-*} \xrightarrow{h\nu} \mathrm{AQDS} + e_{aq}^{\bullet-*} \qquad (10)$$

Hydrated electrons formed under conditions of experiments described in this study would react with about 30% probability with 2,6-AQDS to back form 2,6-AQDS$^{\bullet-}$. The other 70% would react in ca. 1:1 ratio with GlyOR and GlyORH$^+$. This is estimated from the respective rate constants[15] and esters amino group pK_a values of 7.7 (GlyOMe) and 7.8 (GlyOEt). Reactions 8−10 give, therefore, straightforward rational for the observed $^\bullet\mathrm{CH_2COOR}$ radicals genesis.

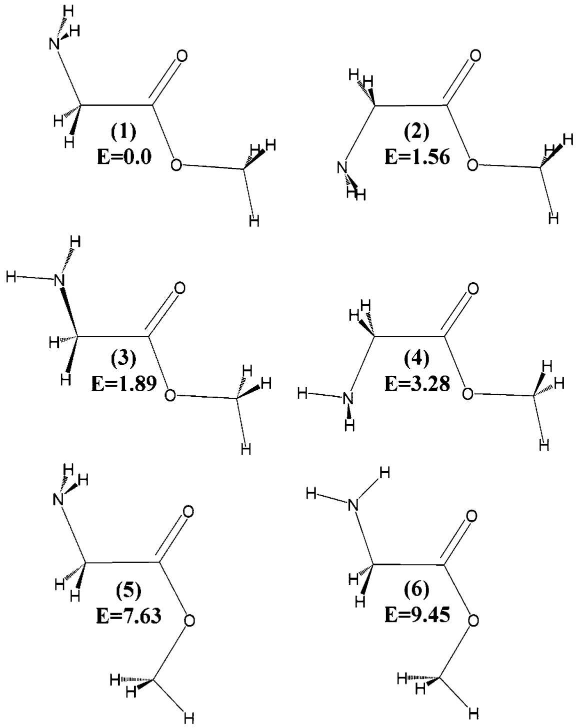

Figure 6. Structure and relative energy E (kJ mol^{-1}) for the six stable conformers of GlyOMe singlet ground state in water calculated with (B3LYP/6-31G(d,p)/SCRF=CPCM).

3. Quantum Chemical DFT Calculations

The quantum chemical calculations were done using the Gaussian 98 package.[41] For the systems under study, geometry optimizations were performed applying the density functional theory (DFT) approach with B3LYP hybrid functionals[42,43] and the standard 6-31G(d,p) basis set. Frequency analyses were used to characterize stationary points and to calculate thermochemical parameters such as zero-point, thermal correction, and activation energy for transformations between different conformers. To investigate the influence of solvent on the molecular structure, electron distribution, and *hfc* constants of radicals studied, geometry optimizations were carried out in water ($\epsilon = 78$) using the CPCM(COSMO)[44,45] self-consistent reaction field (SCRF) model.

First, we will focus on GlyOMe in the singlet ground state. The systematic search both in vacuum and water yields six different stable conformers on the potential surface, shown in Figure 6, with relatively small differences in energy. The geometrical data obtained are similar to the results of Armstrong et al.[46] for glycine and of Fausto et al.[47] for *N*,*N*-dimethylglycine methyl ester. The main framework of GlyOMe (N1−C2−C3−O4−C5) is nearly planar in all conformers, but the structures **(3)**, **(4)**, and **(6)** are slightly bent due to the pyramidality at N1. The transformations between conformers can be easily achieved through rotation against a single C−C or C−N bond. As calculated, the activation energy (barrier to rotation against a C−C bond) between two most stable conformers **(1)** and **(2)** is only 8.8 kJ mol^{-1}, and therefore, the equilibrium between conformers can be adopted at room temperature. On the basis of frequency analysis, the relative concentrations estimated for the four most stable conformers are 1.0:0.08:0.041:0.003 at 298 K (1.0:0.12:0.043:0.007 at 360 K), respectively. These results

TABLE 2: Comparison of *hfc* Constants Calculated (B3LYP/6-31G(d,p)) in Vacuum and Water (SCRF═CPCM) with EPR Parameters of Glycine Ester Radicals Determined by Simulation of the FT EPR Spectra[a]

radical			a_N	$a_{NH(D)}$	a_{CH^1}	a_{CH^2}	$a_{COMe(Et)}$
$^{\bullet}NH-CH_2-CO_2Me$	calc	water	1.401	2.187	4.61*	4.61*	(3)0.008
	exp		1.368	2.279	4.230	4.281	
$^{\bullet}NH-CH_2-CO_2Et$	calc	water	1.401	2.178	4.34*	4.34*	(2)0.013
	exp		1.364	2.280	4.226	4.277	
$NH_2-^{\bullet}CH-CO_2Me(Z)$	calc	vac.	0.791	0.048; 0.472	1.540		(3)0.172
	calc	water	0.665	0.611; 0.688	1.372		(3)0.186
	exp		0.620	(2) 0.515	1.208		(3)0.175
$NH_2-^{\bullet}CH-CO_2Me(E)$	calc	vac.	0.763	0.414; 0.124	1.561		(3)0.111
	calc	water	0.554	0.784; 0.816	1.385		(3)0.123
	exp		0.625	(2) 0.527	1.181		(3)0.127
$NH_2-^{\bullet}CH-CO_2Et(Z)$	calc	vac.	0.800	0.020; 0.461	1.540		(2)0.246
	calc	water	0.642	0.726; 0.668	1.372		(2)0.264
	exp		0.618	(2) 0.515	1.208		(2)0.169
$NH_2-^{\bullet}CH-CO_2Et(E)$	calc	vac.	0.766	0.103; 0.412	1.562		(2)0.160
	calc	water	0.665	0.635; 0.652	1.376		(2)0.187
	exp		0.622	(2) 0.528	1.183		(2)0.124
$^{\bullet}CH_2-CO_2Me$	calc	water			2.204	2.204	(3)0.192
	exp				2.131	2.131	(3)0.157
$^{\bullet}CH_2-CO_2Et$	calc	water			2.198	2.194	(2)0.294
	exp				2.122	2.122	(2)0.161

[a] The *hfc* constants are given in mT. In parenthesis the numbers of equivalent nuclei are indicated. * average value of *hfc* constants for two H atoms.

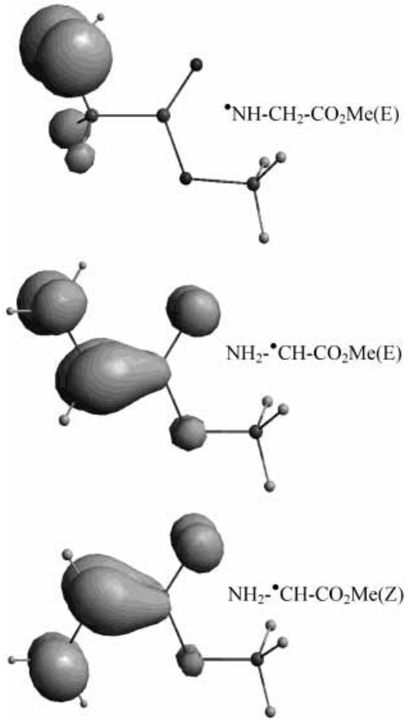

Figure 7. Spin density distribution of some radicals calculated with (B3LYP/6-31G(d,p)/SCRF═CPCM) in water.

allow the conclusion that we can expect two different conformers of the glycine esters radical cations (the aminium radicals) and their successor radicals with detectable concentrations. In the analysis of the FT EPR spectra, we found only one structure of the N-centered aminyl radical, $^{\bullet}NH-CH_2-CO_2R$, but two structures of the glycyl radical, $NH_2-^{\bullet}CH-CO_2R$. This result can be understood by quantum chemical calculations, as we will show in the following.

By DFT calculations, the radical geometry and *hfc* constants were studied for the conformational isomeric structures of the aminyl radicals, glycyl radicals, and alkyl radicals $^{\bullet}CH_2CO_2R$ (also no isomers experimentally observed). The *hfc* constants obtained for the relevant radicals are summarized in Table 2. As calculations have shown, the solvent effect of water on the radical geometry and electron distribution is relatively small in the case of $^{\bullet}NH-CH_2-CO_2R$ and $^{\bullet}CH_2CO_2R$ radicals. Therefore, for these radicals, only results of calculations in water are presented in Table 2. The calculated *hfc* constants, both for R = Me and R = Et, are practically in quantitative agreement with the experiment. The *hfc* constants for the two isomers of $^{\bullet}NH-CH_2-CO_2R$ are so close together that no differences in the experimental spectra can be observed. Additionally, a fast interconversion between the two isomers**,** due to the small activation energy of rotation (about 20 kJ mol^{-1}), averages small differences to the mean values of *hfc* constants.

Substantially different results have been obtained in the case of $NH_2-^{\bullet}CH-CO_2R$ radicals. The effect of solvent is considerable both for the radical structure and for the electron distribution. The two stable isomers (Z) and (E), which correspond to the conformers **(1)** and **(2)** of the singlet ground-state glycine esters, can be localized by geometry optimizations, with the (Z) conformer being 3.9 kJ mol^{-1} more stable. The activation energy to rotation against the C−C bond between isomers (Z) and (E) (about 85 kJ mol^{-1}) is essentially larger than for the neutral molecule, thus preventing fast interchange. The high barrier to rotation is apparently caused by the intramolecular interaction of the unpaired electron on C2 with the nonbonding electron pair on carbonyl oxygen. It follows that, due to the high barrier for interconversion, the exchange between isomers occurs slowly and both isomers can be separately observed in the microsecond time-scale in their FT EPR spectra.

Results of *hfc* constants calculations for different structures of $NH_2-^{\bullet}CH-CO_2R$ radicals, both in a vacuum and water, are also presented in Table 2. As it can be seen, the agreement of calculated with experimentally obtained values could be essentially improved by the consideration of water as a dielectric continuum. An especially strong solvent effect was observed for H atoms on nitrogen. The analysis of molecular structures shows strong distortion from the sp^2 hybridization on the $-NH_2$ group in a vacuum. Thus, the existence of some amount of pyramidality on nitrogen leads to different interactions of these two protons in the β position to the radical center (with the unpaired electron on C2). However, if solvent water is taken into account, the extent of pyramidality has been reduced. In the case of $NH_2-^{\bullet}CH-CO_2R$ in (E) form, the calculated deviations from planarity on $-NH_2$ are about 31° and 10° in vacuum and water, respectively. Moreover, the calculated activation energy for the exchange of protons through a plane between two energetically equivalent pyramidal nitrogen forms is only 5.4 kJ mol^{-1}. It follows that the small distortion in water from sp^2 hybridization on nitrogen and the low barrier of interconversion should lead to two practically equivalent protons, a phenomenon which was actually experimentally observed.

Conclusions

Photooxidation of glycine methyl (ethyl) ester by the triplet state of anthraquinone-2,6-disulfonate in aqueous solution results in the formation of the aminyl radical $^{\bullet}NH-CH_2-CO_2R$ and two conformational isomers (Z and E) of the glycyl radical $NH_2-{}^{\bullet}CH-CO_2R$. The *hfc* patterns of these radicals were unambiguously resolved from the FT EPR spectra obtained on 0.1 μs time-scale at room temperature. (Z) and (E) isomeric forms of the glycyl radicals were present in about 7:3 ratio, and no interconversion was observed in the time of up to about 1 μs. The results of quantum chemical DFT calculations strongly support the experimental observations of the existence of (Z) and (E) conformers. Consideration of solvent water as dielectric continuum remarkably improved agreement between calculated and experimentally determined EPR parameters.

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References and Notes

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(8) Yields of radicals in primary processes vary depending on the type of oxidizing agent. For example, oxidation of glycine anions by hydroxyl radicals leads to decarboxylation with 22% efficiency. Other products were glycyl (37%) and aminyl radicals (22%).[9, 10]

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