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COMMUNICATION

Direct EPR observation of the aminomethyl radical during the radiolysis of glycine

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Aminomethyl ($^{\bullet}CH_2NH_2$) and $H_2N{-}^{\bullet}CH{-}CO_2^-$ radicals were detected in time-resolved EPR experiments following the reaction of $^{\bullet}OH$ radicals with glycine anions.

Oxidation of amino acid residues in proteins is a main focus of interest in oxidative stress.[1] Oxidation of glycine, the simplest amino acid, usefully models the degradation of key organic complexants in the strongly basic media of nuclear-waste storage tanks.[2] The mechanism of free-radical oxidation of glycine anions is the subject of this communication. In particular, we report here the detection and identification, by time-resolved electron paramagnetic resonance (EPR) spectroscopy, of two carbon-centered radicals recently suggested[3] to participate in the mechanism of the radiolytic oxidation of the glycine anion. The new observations provide direct evidence implicating these C-centered radicals in the primary radiolytic processes.

In 1971 the $H_2N{-}^{\bullet}CH{-}CO_2^-$ radical was detected by EPR in the steady-state electron irradiation of glycine in N_2O–saturated aqueous solutions at high pH.[4] In highly basic solutions, no other radicals were seen on this time scale (estimated radical lifetimes were in the 100's of microseconds to be observable by this technique). However, in 1985, extensive CO_2 was detected[5] in gamma-irradiated basic aqueous solutions of glycine, and this large yield was rationalized as being accompanied by extensive amounts of $^{\bullet}CH_2NH_2$. This observation and conclusion appeared to be in conflict with steady-state EPR experiments, using both radiolytic[4] and $Ti^{3+}{-}H_2O_2$ initiation,[6–10] in which no $^{\bullet}CH_2NH_2$ radicals were reported following the oxidation of glycine anions by $^{\bullet}OH$. This conflict was resolved by postulating the participation of reaction (1),[5]

$$^{\bullet}CH_2NH_2 + H_2N{-}CH_2{-}CO_2^- \longrightarrow CH_3NH_2 + H_2N{-}^{\bullet}CH{-}CO_2^- \quad (1)$$

which converts the highly reactive aminomethyl radical ($^{\bullet}CH_2NH_2$) into the less reactive $H_2N{-}^{\bullet}CH{-}CO_2^-$ radical.

Recently a comprehensive mechanism[3] was proposed that attempted to reconcile the observation of $^{\bullet}OH$ oxidation of glycine anions on the short-time scale (10's of nanoseconds) with steady-state observation of large CO_2 yields. The short-time experiments used the optical detection of products generated by scavenging of the radicals formed following the direct $^{\bullet}OH$ attack on glycine anions. The behavior was comprehensively explained by a scheme that involved about $\frac{2}{3}$ of the $^{\bullet}OH$ yield generating $^{\bullet}CH_2NH_2$ and CO_2, through an intermediate zwitterion radical $H_2N^{\bullet+}{-}CH_2{-}CO_2^-$. It was also proposed that the remaining $\frac{1}{3}$ of the $^{\bullet}OH$ radicals generated the oxidizing aminyl radical, $HN^{\bullet}{-}CH_2{-}CO_2^-$. Evidence for the oxidizing $HN^{\bullet}{-}CH_2{-}CO_2^-$ radical was seen in scavenging experiments that optically monitored the oxidation of hydroquinone in pulse radiolysis.[3]

The relative yield of $\frac{2}{3}$ for reduced products (such as the methyl viologen radical cation) from the scavenging reactions matched within experimental error the steady-state CO_2 yields when the glycine anion concentrations were in the 1–5 mM range.[3] This strongly suggested that there was a direct connection between the $^{\bullet}CH_2NH_2$ radical being the major reducing radical in the oxidation of glycine anions and the CO_2 yields. On the contrary, if the $H_2N{-}^{\bullet}CH{-}CO_2^-$ radical were being formed, it would not be expected to decarboxylate.

The original EPR experiments ($H_2N{-}^{\bullet}CH{-}CO_2^-$ detection) had the disadvantage of only being able to detect the longer lived radicals, and the nanosecond optical experiments ($^{\bullet}CH_2NH_2$ assignment) were limited to indirect scavenging observations.[3] In order to gain insight into how these two diverse sets of observations and interpretations could be resolved, we looked at pulse radiolysis with time-resolved EPR detection. The experimental conditions were taken as close as possible to the optical experiments. The concentration of glycine was either 10 or 100 mM, and the pH of the N_2O-saturated aqueous solutions was usually 10.6. This particular pH was chosen because it is intermediate between the pK_a of $^{\bullet}OH$ at 11.8 and the pK_a of the glycine zwitterion at 9.6. Solutions were irradiated with 2.8 MeV electrons from a Van de Graaff accelerator. The detection system has been previously described.[11]

Some key results are shown in Fig. 1 which shows the kinetic profile of the line for $H_2N{-}^{\bullet}CH{-}CO_2^-$ at 12.70 G above the center of the spectrum. Other lines were found corresponding to hyperfine splittings of $a(NH') = 3.39$ G, $a(NH'') = 2.90$ G, $a(CH) = 13.70$ G, and $a(N) = 6.10$ G with an isotropic g-value of 2.00347. These hyperfine splittings are within experimental error of those originally reported for $H_2N{-}^{\bullet}CH{-}CO_2^-$ in radiolysis experiments.[4] Fig. 1b shows a kinetic trace of one of the lines of $^{\bullet}CH_2NH_2$ (at 20.28 G above the center of that spectrum). Other lines at field offsets that match the hyperfine splittings $a(CH_2) = 15.35$ G, $a(NH_2) = 4.60$ G, and $a(N) = 4.93$ G were followed in the same manner. The isotropic g-value was 2.00289 for this radical. These parameters are consistent with the previously observed aminomethyl radical formed in the radiolysis of methylamine by $^{\bullet}OH$ in basic aqueous solutions.[4] This radical was not reported previously in the steady-state EPR oxidation of glycine anions by $^{\bullet}OH$.[6–10,12] The time-resolved traces showed clearly that the low-field lines were in emission and the high-field lines were in enhanced absorption for both $H_2N{-}^{\bullet}CH{-}CO_2^-$ and $^{\bullet}CH_2NH_2$ (as expected for CIDEP produced by the radical pair mechanism in random encounters). There was no significant difference in the time behavior of $^{\bullet}CH_2NH_2$ at 10 and 100 mM glycine, see eqn. (1).

The yields of radicals were measured by a comparison of the integrated line intensities, extrapolated to the time of the radiolysis pulse, with the intensity so determined for the $SO_3^{\bullet-}$ radical. The effect of CIDEP was removed either by using lines near the center of the spectrum or by taking an average of symmetrically located lines. The $SO_3^{\bullet-}$ radical is commonly used as an EPR standard,[13] and its yield in N_2O-saturated aqueous solutions of SO_3^{2-} is equal to the full yield of $^{\bullet}OH$. The full yield of $^{\bullet}OH$ in this situation is equal to its yield from the primary radiolysis of water plus that from the conversion of the hydrated electron (e_{aq}^-) into $^{\bullet}OH$ through the reaction of e_{aq}^- with N_2O. The values determined for the yields of $H_2N{-}^{\bullet}CH{-}CO_2^-$ and $^{\bullet}CH_2NH_2$ by this new method are 50 and 28%, respectively, of the full $^{\bullet}OH$ yield.

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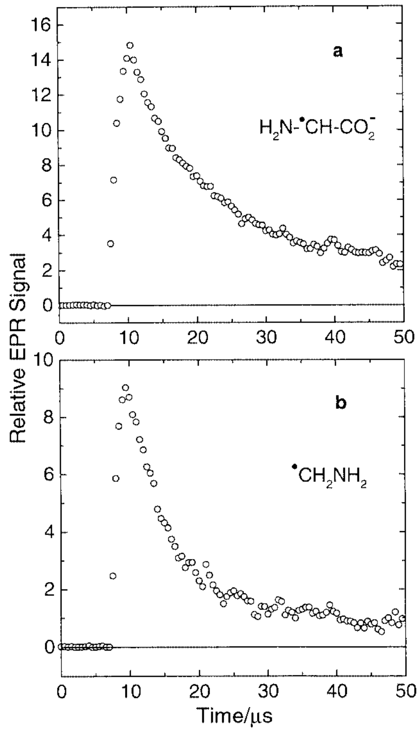

Fig. 1 Time-dependent EPR signals following 1 µs electron pulses to an aqueous N_2O-saturated solution of 100 mM glycine at pH 10.6. Time behavior of EPR lines (a) from the H_2N–•CH–CO_2^- radical at 12.70 G above the center and (b) from the •CH_2NH_2 radical at 20.28 G above that center. The microwave detector is gated during the electron pulse, and there is a dead period of about 1 µs after the radiolysis pulse. The EPR signal shows no significant further delay. The signal channel has a 0.93 µs time constant in these kinetic traces.

Simulations using stochastic kinetics[14] with the rate constants from the recently proposed scheme,[3] show that it is unlikely that a sufficiently high concentration of H_2N–•CH–CO_2^- can be produced quickly enough to account for the time profiles such as the one in Fig. 1a. On the other hand, the EPR experiments do confirm two aspects of the recently proposed scheme.[3] First, the •CH_2NH_2 radical was directly observed in the radiolytic oxidation of glycine, and, second, the total yield (78%) of observed radicals is less than the full •OH yield. The difference from 100% is believed to be larger than the expected error so the latter finding leaves open the possibility that there is an oxidizing HN•–CH_2–CO_2^- radical (undetected by EPR, possibly because of a large line width) as proposed in the scheme.[3] In support of this idea, a similar EPR study on methylamine itself found •CH_2NH_2 to be only about 50% of the total •OH yield with a larger missing fraction attributable to •$NHCH_3$.

We have also performed several Density Functional Theory (DFT) calculations in support of the conclusions of this work. Structures were optimized using a hybrid functional, B3LYP,[15] with a modest polarized split-valence 6-31G* basis set.[16] This procedure has been shown to provide, for a roughly comparable computational cost, geometries and vibrational frequencies in markedly closer agreement to experiment than those obtained at the Hartree–Fock level. Solvent effects were introduced using the self-consistent isodensity version of the polarized cavity model (SCIPCM)[17] with an isodensity contour of 0.001 au and a relative permittivity appropriate for water. Hyperfine coupling constants were obtained at the B3LYP/6-31G* geometries using the same functional but with a larger basis set specifically designed for the economical recovery of magnetic interactions.[18] All calculations were performed with a modified version of the GAUSSIAN94 suite of electronic structure programs.[19]

We find that the zwitterionic radical formed upon electron loss from the glycine anion is best described as an acyloxyl radical in the gas phase. In aqueous solution (*i.e.* upon structural reoptimization within the SCIPCM approach) this radical develops a very long (~2 Å) CC bond to give a structure which is best described as a weak complex between CO_2 and •CH_2NH_2. Energetically, in aqueous solution, the complex lies only 2.2 kcal mol^{-1} below the separately solvated CO_2 and •CH_2NH_2. The implication is that in solution electron loss from the glycine anion leads to rapid decarboxylation. The carbon-centered radical anion, H_2N–•CH–CO_2^-, formed from the glycine anion by H-abstraction is predicted to be 2 kcal mol^{-1} (in the gas phase) more stable than the N-centered radical anion, HN•–CH_2–CO_2^-, and this value increases to 10.2 kcal mol^{-1} in aqueous solution. Thus if the missing yield is from the HN•–CH_2–CO_2^- radical, its formation is kinetically controlled.

The observations reported herein show that two different C-centered radicals are formed very early in the radiolysis of aqueous solutions of glycine anions and that the sum of their yields falls short of the yield of •OH radicals.

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