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Absolute Rate Constants and Yields of Transients from Hydroxyl Radical and H Atom Attack on Glycine and Methyl-Substituted Glycine Anions

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The reaction of the amino acid anions, $R_2N-CR_2-CO_2^-$ (R = H or methyl), with $^{\bullet}OH$ radicals and $H^{\bullet}$ atoms was quantified with respect to the site of attack, the respective absolute rate constants, and the yields of the primary transients generated in these processes. The method applied was pulse radiolysis with time-resolved optical detection. Specifically investigated amino acids were glycine, alanine, α-methylalanine and *N*,*N*-dimethylglycine. Absolute overall rate constants, as determined from the growth of UV absorptions and competition with carbonate, ranged from $(1.7-3.6) \times 10^9$ M^{-1} s^{-1} for the reaction of $^{\bullet}OH$ with the anions of these amino acids, and $(0.1-1) \times 10^8$ M^{-1} s^{-1} for the corresponding reaction with the respective zwitterions. $H^{\bullet}$ atoms react with amino acid anions containing $C_\alpha-H$ bonds with a rate constant of 1.4×10^8 M^{-1} s^{-1}, whereas $k < 10^7$ M^{-1} s^{-1} was estimated for the reaction with α-methylalanine. The primary transient radicals from these reactions include aminyl radicals ($RN^{\bullet}-CR_2-CO_2^-$), α-amino-α-carboxyalkyl radicals, $R_2N-C^{\bullet}R-CO_2^-$, α-aminoalkyl radicals, $R_2N-C^{\bullet}R_2$, and ($^{\bullet}CH_2-$) type side-chain radicals (for $R = CH_3$ compounds). The yields of the reducing species (all but aminyl) were determined via titration with electron acceptors of different and thus distinguishing reduction potentials, namely, 4-carboxybenzophenone, methyl viologen, and hexacyanoferrate-III. On the basis of the overall rate constants and the yields of the various transients, partial rate constants were evaluated for the attack of $^{\bullet}OH$ at $C_\alpha-H$, at the lone electron pair at nitrogen, and at the more remote methyl groups. The results substantiate earlier conclusions that the amino nitrogen is indeed the preferred site of oxidative attack, but also that substantial amounts of $R_2N-C^{\bullet}R-CO_2^-$ type radicals are formed via direct abstraction of hydrogen from the $C_\alpha-H$ bond. Trends and individual data are discussed in the light of structure and substitution pattern of the amino acids investigated.

Introduction

The degradation of natural compounds in aqueous environments is often induced by free radicals. Among these, the highly reactive hydroxyl radical ($^{\bullet}OH$) is a particularly aggressive one. It can be formed, for example, in the radiolysis of water, via Fenton chemistry, and also as an intermediate in numerous other chemical and biochemical reactions. Another reactive species, often produced simultaneously with $^{\bullet}OH$, e.g., in radiolysis, is the H atom. There has been a long-standing interest in the reaction mechanisms of these two radicals, particularly with compounds having multiple functional groups as targets for the attack. These include amino acids, which are essential for life and have importance in various industries.

In recent papers on this topic we already focused attention on processes induced by $^{\bullet}OH$ radical oxidation of deprotonated amino acids of the general type $R_2N-CR_2-CO_2^-$ (R = H or CH_3).[1,2] Complementary experiments were also done using the benzophenone triplet as a specific one-electron oxidant.[3] One major aim has been the identification and quantification of transient radicals formed in these processes, and their physicochemical properties. This has mainly been achieved by applying selective redox scavengers. Kinetic information on the reactions of the transients was obtained from time-resolved investigations using the techniques of pulse radiolysis and laser flash photolysis. In addition, CO_2 was measured as a characteristic end-product in γ-radiolysis. The results revealed a much more complex mechanism for the radical-induced degradation of amino acids than assumed earlier.[4−6]

Scheme 1 shows all proven and postulated primary reactions of the $^{\bullet}OH$ radical with amino acid anions. The free valences at nitrogen and the central C_α indicate H or CH_3 substitution. A variety of radicals can simply be formed by H abstraction and stoichiometric loss of water. One pathway involves a transient formed by one-electron oxidation of nitrogen followed by its decarboxylation. Electron loss from the nonbonding electron pair on nitrogen, as shown in reaction 4, results in the formation of the aminium radical zwitterion **3** (For all transients of the same type, the bold number is used throughout the paper irrespective of hydrogen or methyl substitution.) which resides transitorily in a solvent cage in close proximity to the conjugate OH^-. (Such a transient cage seems more realistic than a three-electron-bonded radical anion, $HO\therefore NR_2-CR_2-CO_2^{-}$.[7]) Radical **3** is highly unstable. It is prone to a very fast heterolytic C−C bond rupture (reaction 9, with $k \sim 10^{11}$ s^{-1} i.e., $t_{1/2}$ in the picosecond range[3] to produce CO_2 and the α-aminoalkyl radical **6**. Competitively, in derivatives where H atoms are present at the α-C center or at N, the strong base OH^- located in the cage serves as a proton acceptor from those positions. Such a proton-transfer results in a different type of reducing radical, **2**, or the

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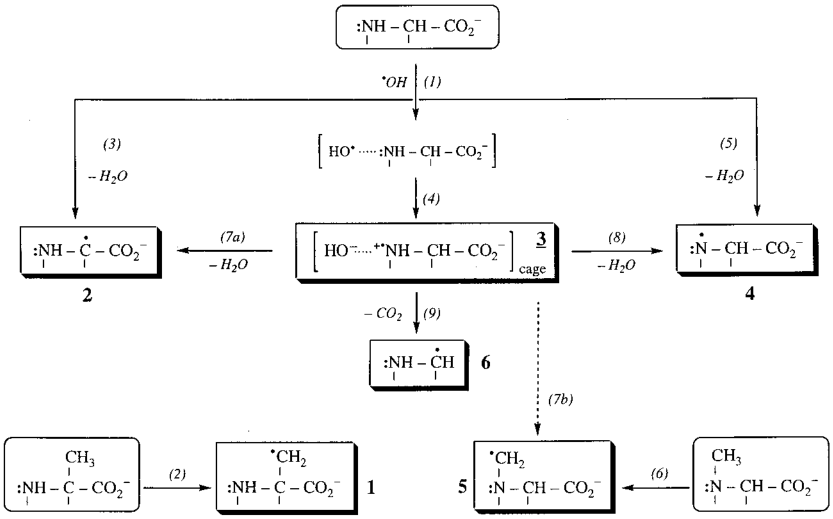
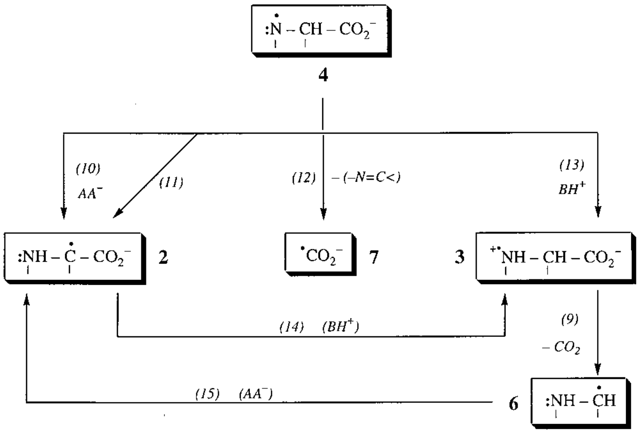
SCHEME 1: Reaction of Amino Acid Anion (AA$^-$) with $^{\bullet}$OH Radicals (Equations 1−6) and Follow-up Processes (Equations 7−9)



SCHEME 2: Reactions of Aminyl Radical 4 BH$^+$: Proton Donor (e.g., Phosphate, Amino Acid Zwitterion AA$^{\pm}$). AA$^-$: Amino Acid Anion



oxidizing aminyl radical **4** in reactions 7a and 8, respectively.[3] These two radicals can, in principle, also be formed via direct H atom abstraction by $^{\bullet}$OH (reactions 3 and 5, respectively). Like **6**, the also C-centered α-amino-γ-carboxyalkyl radical anion **5**, derived from *N*,*N*-dimethylglycine, may be formed via direct H atom abstraction (reaction 6), or by proton transfer to OH$^-$ in the cage (reaction 7b). For the formation of the side-chain C-centered radical **1**, only a direct H atom abstraction (reaction 2) seems feasible.

Scheme 2 shows secondary reactions of the aminyl radical **4**, which is more stable than the extremely short-lived aminium radical **3**. A first-order β-cleavage (homolytic C−C bond rupture) leads to liberation of $^{\bullet}CO_2^-$ (reaction 12). This typically occurs on the microsecond to millisecond time scale.[2] Competing processes of **4** include a bimolecular reaction with the amino acid anions to produce the more stable radical **2** (reaction 10). The latter may also result from a monomolecular transformation of **4** via a 1,2-H atom shift (reaction 11); however, this pathway is slow and, therefore, only of minor, if any significance.[2] Most important is a reprotonation of **4** which can be achieved by any proton donor BH$^+$ (which can be any buffer, including the amino acid's own zwitterion) in reaction 13 to yield the aminium radical **3**. This process can be effective even in basic solution due to the fast and irreversible decarboxylation of **3**. Furthermore, at high amino acid and/or proton donor concentrations, radicals **2** and **6** become involved in reactions 14 and 15. Both are, however, very slow. Nevertheless, together with reaction 9 they establish a chain mechanism for generation of CO_2.[1]

Schemes 1 and 2 do not show the radical−radical termination reactions, which produce stable nonradical products other than CO_2.[4,5] For γ-radiolysis such reactions occur typically on a ms time scale, and an even longer one for thermally initiated oxidations. The fastest radical−radical processes are likely to be the cross reactions between the oxidizing radical **4** and the reducing ones, **2**, **6**, and **7** leading to the repair of radical **4** into its parent amino acid anion. This is illustrated for the reaction of glycine-derived radicals in eq 16. The other product in this reaction, $HN{=}CH-CO_2^-$, hydrolyses into glyoxalic acid (reaction 17) which has, in fact, been observed in the product analysis.[1]

$$H_2N-{}^{\bullet}CH-CO_2^-\ (\mathbf{2}) + H^{\bullet}N-CH_2-CO_2^-\ (\mathbf{4}) \rightarrow H_2N-CH_2-CO_2^- + HN{=}CH-CO_2^- \quad (16)$$

$$HN{=}CH-CO_2^- + H_2O \rightarrow NH_3 + O{=}CH-CO_2^- \quad (17)$$

The preceding paragraphs summarize the results of our studies of hydroxyl radical reactions with glycine derivatives by redox titration methods and CO_2 yield determinations. Until very recently, ESR studies had identified only the C-centered $NH_2-{}^{\bullet}CH-CO_2^-$ radical **2**.[8−10] However, Hug and Fessenden[11,12] have now confirmed, for the first time by ESR, that the other two primary radicals identified in our scavenging experiments, namely, $NH_2-{}^{\bullet}CH_2$ (**6**) and $^{\bullet}NH-CH_2-CO_2^-$ (**4**), are indeed also formed as primary intermediates. In addition, the presence of $^{\bullet}CO_2^-$ has been reported in another ESR study of glycine[13] providing independent evidence for reaction 12 in our mechanism (Scheme 2). Likewise, the results of Hug and Fessenden are consistent with the key reactions in Scheme 1, namely, reaction 4 followed by 9, 3, or 4 + 7a, and 5 or 4 + 8. The most significant common conclusion from these new ESR results and our own work is that not only the $C_\alpha-H$ bond but also the amino function, especially in its deprotonated form, are prime centers for an attack by the hydroxyl radical. This, in turn, nicely corroborates the results obtained for the $^{\bullet}$OH-induced oxidation of simple amines.[4] It may be noted that attack on the N-center also occurs in the reaction of the t-ButO$^{\bullet}$ alcoxyl radical with Me_2NH.[14] Here, the yield of the primary aminyl radicals is, in fact, larger than that of the C_α-centered.

TABLE 1: Characteristics of the Specific Scavengers Used, and Their Reactivity toward Radicals Derived from Amino Acids Anions ($R = CH_3$ or H)[a]

scavenger	measurement	λ_{max}/nm ($\epsilon/M^{-1}\,cm^{-1}$)	$E°$/V vs NHE	$^{\bullet}CR_2{-}NR_2$[b]	$^{\bullet}CO_2^-$	$NR_2{-}^{\bullet}CR{-}CO_2^-$	$^{\bullet}CH_2{-}NR{-}CR_2{-}CO_2^-$	$^{\bullet}NR{-}CR_2{-}CO_2^-$
$Fe(CN)_6^{3-}$	bleaching of $Fe(CN)_6^{3-}$	**420** (1020)	$[Fe(CN)_6^{3-}/Fe(CN)_6^{4-}]$ $+$ **0.36**	+	9×10^8 $M^{-1}\,s^{-1}$[c]	+	$10^6{-}10^7\ M^{-1}\,s^{-1}$	−
MV^{2+}	formation of $MV^{\bullet+}$	**600** (12820)	$[MV^{2+}/MV^{\bullet+}]$ $-$ **0.45**	+	1×10^{10} $M^{-1}\,s^{-1}$[d]	+	−	−
CB^-	formation of $^{\bullet}CB^{2-}$	**660** (7660)	$[CB^-/^{\bullet}CB^{2-}]$ $-$ **1.13**	+	3.4×10^7 $M^{-1}\,s^{-1}$[e]	−	−	−
H_2Q	formation of $Q^{\bullet-}$	**427** (7200)	$[Q^{\bullet-}/Q^{2-}]$ $+$ **0.023**	−	−	−	−	$\approx 10^8\ M^{-1}\,s^{-1}$[f]

[a] Specific rate constants are listed numerically; close-to or diffusion-controlled rate constants are marked with “+”, no reaction with “−”. [b] Also valid for $^{\bullet}CH_2{-}NR{-}CR_2{-}CO_2^-$ radical. [c] From ref 18. [d] From ref 18. [e] From ref 19. [f] At pH 11, from refs 1 and 2.

During the course of our studies with glycine and the methylated derivatives, some anomalies and gaps became apparent with respect to the rate constants for their reactions with $^{\bullet}OH$. One aim of this study was, therefore, to obtain a consistent set of rate measurements for compounds with methyl substitution at both the α-C and N positions under comparable conditions. At the same time, the effects of methyl substitution on the relative yields of the different primary radicals (**1**−**6** shown in Scheme 1) was examined. Rates and yields were also examined for reactions induced by $H^{\bullet}$ atoms since no data were available so far for the interaction of this species with amino acid anions. H atoms are produced simultaneously with hydroxyl radicals during the radiolysis of aqueous solutions and exhibit similar properties to $^{\bullet}OH$ with respect to abstraction reactions. However, none of the possible radicals from the H atom reaction with the amino acids is expected to exhibit any characteristic optical absorption outside the region ($\lambda < 300$ nm) where spectral overlap hampers unambiguous identification.[15] Yield and kinetic measurements have, therefore, been complemented by redox scavenging methods analogous to those described in our previous studies on the $^{\bullet}OH$-induced oxidation.[1−3]

Experimental Section

The experimental procedures concerning preparation of solutions, irradiation, and analysis of data were practically the same as described previously.[1,2] Therefore, only some essential features will be repeated here.

All investigations have been conducted in aqueous solutions with water purified by the Serv-A-Pure Co. system. The amino acids and other chemicals were used as received from the vendors (Aldrich, Flucka, and Sigma). Adjustments of pH were achieved by adding appropriate amounts of $HClO_4$ or NaOH.

Pulse radiolysis was performed with an 8 MeV Titan Beta model TBS-8/16-1S LINAC. Typical pulse lengths were 2.5−10 ns, with doses of 2−10 Gy. Solutions were generally deaerated and saturated with N_2O. Under those conditions approximately 90% of all primary radicals available for reaction with the amino acid solutes were $^{\bullet}OH$ radicals. The remaining ∼10% were $H^{\bullet}$ atoms and for their yield a value of $G(H^{\bullet}) = 0.06\ \mu M\ J^{-1}$ was applied throughout the study. However, for the more abundant $^{\bullet}OH$ radicals allowance was made for the fact that the yield actually scavenged depended on the solute concentration [S] and the rate constant of its reaction with substrate k_S. $G(^{\bullet}OH)$ was accordingly calculated using formula I.[16]

$$G(^{\bullet}OH) = \{5.2 + 3.0\,(k_S[S]/4.7 \times 10^8)^{1/2}/(1 + (k_S[S]/4.7 \times 10^8)^{1/2})\}0.1036\ \mu M\ J^{-1} \tag{I}$$

The total concentration of water radicals per typical pulse in N_2O-saturated systems was in the range of $(1{-}6) \times 10^{-6}$ M. Dosimetry was performed with thiocyanate solutions as described earlier.[17]

The chemicals used for quantitative yield determinations via redox titration were methyl viologen (MV^{2+}), $Fe(CN)_6^{3-}$, 4-carboxybenzophenone (CB^-), and hydroquinone (H_2Q).[1−3] Columns 1−4 in Table 1 summarize the relevant data, i.e., the reduction potentials (vs NHE), as well as λ_{max} and the molar extinction coefficients ϵ at λ_{max} of the optically absorbing species traced.

Absolute error limits for radiation chemical experiments are generally considered to be about ±10%. They apply also to our present set of data unless specifically noted. All experiments have been conducted at room temperature.

Throughout the paper we frequently use the following abbreviations: AA for amino acid, AA^- for its anionic form, and $AA^{\pm}$ for the zwitterionic form, Gly for glycine ($H_2N{-}CH_2{-}CO_2H$), Ala for alanine ($H_2N{-}CHMe{-}CO_2H$), MeAla or α-methylalanine for 2-aminoisobutyric acid ($H_2N{-}CMe_2{-}CO_2H$), and *N*,*N*-Me_2Gly for *N*,*N*-dimethylglycine ($Me_2N{-}CH_2{-}CO_2H$).

Results and Discussion

1. Reaction Rate Constants with $^{\bullet}OH$ Radicals. The aliphatic amino acids investigated in this study show two acid−base dissociation equilibria with $pK_1 \sim 2.3$, eq 18, and $pK_2 \sim 10$, eq 19. The overall rate constants for the reactions of hydroxyl radicals with the amino acids in their zwitterionic and anionic forms $k(\cdot OH,AA^{\pm})$ and $k(\cdot OH,AA^-)$ (reactions 20 and 21) were, therefore, determined at pH 4.7 and 10.5−11, respectively.

$$^{+}HNR_2{-}CR_2{-}CO_2H \leftrightarrow {}^{+}HNR_2{-}CR_2{-}CO_2^- + H^+ \tag{18}$$

$$^{+}HNR_2{-}CR_2{-}CO_2^- \leftrightarrow NR_2{-}CR_2{-}CO_2^- + H^+ \tag{19}$$

$$^{\bullet}OH + {}^{+}HNR_2{-}CR_2{-}CO_2^- \rightarrow \text{products} \tag{20}$$

$$^{\bullet}OH + NR_2{-}CR_2{-}CO_2^- \rightarrow \text{products} \tag{21}$$

This ensured selective determination of both rate constants. At the slightly acidic pH of 4.7, the concentration of the fully protonated cationic form, $^{+}HNR_2{-}CR_2{-}CO_2H$, is negligible and that of the much faster reacting anionic form, $NR_2{-}CR_2{-}CO_2^-$, is also too low for any competition with reaction 20. For accurate determination of $k(\cdot OH,AA^-)$ the pH was kept in the range of 10.5−11 in order to push equilibrium 19 as much as possible to the right side, while still not allowing appreciable dissociation of $^{\bullet}OH$ radicals (pK_a 11.9).[20] Also, only the actual concentrations of the anionic $NR_2{-}CR_2{-}CO_2^-$, as calculated from the respective pK_a's and the pH of the solution, were used.

TABLE 2: Absolute Rate Constants for $^{\bullet}$OH Radicals Reaction with Amino Acids in Zwitterionic and Anionic Form and for H Atoms with Amino Acid Anions

amino acid	pK_1	pK_2	pK_2^{eff}	$k(^{\bullet}OH,AA^{\pm})/M^{-1}\ s^{-1}$	$k(^{\bullet}OH,AA^{-})/M^{-1}\ s^{-1}$	$k(H^{\bullet},AA^{-})/M^{-1}\ s^{-1}$
glycine (Gly) $NH_2-CH_2-CO_2^-$	2.34	9.60	7.1	$(8.9 \pm 0.1) \times 10^{6}$ [a] [1.7×10^{7}, pH 5.9, ref 22]	$(2.9 \pm 0.1) \times 10^{9}$ [a] $(2.7 \pm 0.2) \times 10^{9}$ [b] [5.3×10^{9}, pH 10, ref 23; 1.9×10^{9}, pH 9.6, ref 22]	1.2×10^{8} [a] $(1.0 \pm 0.1) \times 10^{8}$ [d] $(1.7 \pm 0.2) \times 10^{8}$ [e]
alanine (Ala) $NH_2-CH(CH_3)-CO_2^-$	2.34	9.69	8.4	$(8.3 \pm 0.4) \times 10^{7}$ [a] [5.2×10^{7}, pH 6, ref 24; 6.5×10^{7}, pH 7.4, ref 25; 7.7×10^{7}, pH 5.8, ref 22]	$(1.78 \pm 0.03) \times 10^{9}$ [a] $(1.70 \pm 0.02) \times 10^{9}$ [b] [7.5×10^{8}, pH 9.75, ref 24]	n.d.[f]
2-methylalanine (MeAla) $NH_2-C(CH_3)_2-CO_2^-$	2.36	10.21	8.9	$(1.14 \pm 0.04) \times 10^{8}$ [c]	$(2.4 \pm 0.1) \times 10^{9}$ [a] $(2.4 \pm 0.2) \times 10^{9}$ [b]	$<10^{7}$ [d]
N,*N*-Dimethylglycine $N(CH_3)_2-CH_2-CO_2^-$	2.08	9.80	7.4	$(1.45 \pm 0.03) \times 10^{7}$ [a]	$(3.7 \pm 0.2) \times 10^{9}$ [a] $(3.5 \pm 0.4) \times 10^{9}$ [b]	$(1.4 \pm 0.1) \times 10^{8}$ [d]

[a] Direct measurement of products formation kinetics. [b] Competition method relative to carbonate ion. [c] Competition method relative to $Fe(CN)_6^{4-}$. [d] Measured in N_2O-saturated, pH 9−11 aqueous solution containing 2 M 2-methyl-2-propanol, 10 mM amino acid, 0.5 mM MV^{2+}. [e] Measured in N_2O-saturated, pH 9−11 aqueous solution containing 0.2 M DMSO, 10 mM amino acid, 0.5 mM MV^{2+}. [f] n.d. = not determined.

The pulse radiolysis measurements of the rate constants were performed in N_2O-saturated solutions by three methods: (i) direct measurements of the pseudo-first-order formation kinetics of the fastest growing absorption (usually at 250−260 nm) at different AA concentrations. This method could be applied for all AA^- and $AA^{\pm}$ except the zwitter anion of α-methylalanine, for which the radical products showed practically no optical absorption at $\lambda > 240$ nm. (ii) For $MeAla^{\pm}$ in acidic solutions, instead, competition experiments with $Fe(CN)_6^{4-}$ were performed. (iii) For all of the amino acids in basic solutions, complementary competition experiments were conducted with carbonate as $^{\bullet}$OH scavenger. The following reference values[20] were used for the competitions: $k(^{\bullet}OH+Fe(CN)_6^{4-}) = 1.0 \times 10^{10}\ M^{-1}\ s^{-1}$ and $k(^{\bullet}OH+CO_3^{2-}) = 3.9 \times 10^{8}\ M^{-1}\ s^{-1}$. (Carbonate ion concentrations were kept above 0.1 mM and corrected for the amount of CO_2 in water from air.[21]) The results for $k(\cdot OH,AA^{\pm})$ and $k(\cdot OH,AA^{-})$ are listed in Table 2.

The most important feature of the results is that $k(\cdot OH,AA^{\pm})$ is always lower than $k(\cdot OH,AA^{-})$. The difference becomes smaller with the number of methyl groups substituted at the α-carbon. For Gly and the *N*,*N*-dimethylglycine, the ratio is about 300. The comparatively low values of $k(\cdot OH,AA^{\pm})$ must be attributed to a greatly reduced reactivity of the protonated amino group, which can no longer undergo electron transfer and from which H abstraction by $^{\bullet}$OH will be impeded by a high dissociation energy of the H−N bonds.[26] In addition, in the zwitterion the C_α−H bonds are also stronger (ca. 55 kJ mol^{-1}) than in the anion, due to loss of the stabilization of the radical by the nitrogen lone pairs.[26] Concerning the amino acid anions, the agreement between the direct and the carbonate competition methods is excellent. Agreement between the present results and those available in the literature, particularly those based on the mechanistically more complex thiocyanate competition method, is only fair. In any case, some anomalous ambiguities for certain substitution patterns seen in the earlier data are now removed, and the present results also fill some obvious gaps in the literature. A more detailed discussion of the effects of substitution and of the rate constants for reactions at specific sites within the various amino acids will be presented below, after the determinations of the relative yields of the different primary radicals have been reported.

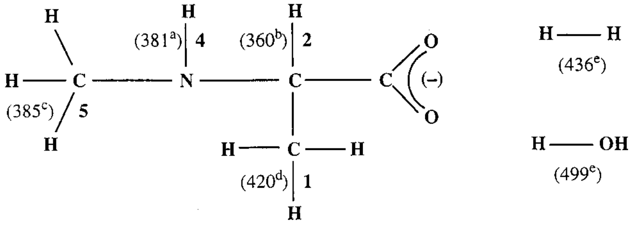
Knowing the absolute rate constants it is now possible to evaluate the pHs at which 50% of hydroxyl radicals would react with the amino acid in its anionic form. These are denoted as pK_2^{eff} and are listed together with the respective pK_2 values in Table 2. Because of the dramatic decrease of $k(\cdot OH)$ upon protonation of the amino group, pK_2^{eff} can be as much as 3 pH units below pK_2 and approach values near 7. That makes the mechanism for amino acid anions discussed in this and earlier studies quite relevant also for biological systems.

2. Reaction Rate Constants with H Atoms. As pointed out earlier, H atoms constitute about 10% of the reactive species in irradiated N_2O-saturated aqueous solutions. To identify any possible contributions to the radical yields by H atoms it is, in the first instance, necessary to know their rate constants for reaction with the AAs. Literature values of $k = (7-8) \times 10^{4}\ M^{-1}\ s^{-1}$ were given for the reaction of $H^{\bullet}$ with glycine at pH 1 and 7,[20] but no value has been reported for the reaction with the anionic form. Because of the decrease of the C_α−H bond energy from 415 to 360 kJ mol^{-1} upon amino group deprotonation,[26] $k(\cdot H,AA^{-})$ for reaction 22 is expected to be considerably larger.

$$H^{\bullet} + NR_2-CRH-CO_2^{-} \rightarrow NR_2\text{-}^{\bullet}CR-CO_2^{-} + H_2 \quad (22)$$

Concerning other possible reactions, an electron transfer from AA^-, in analogy to reaction 4, can clearly be ruled out because of the low reduction potential of the H atom. Bond energy considerations for those anions possessing at least one H atom at the C_α position, confirm that this hydrogen atom is indeed the most labile. That point is illustrated by the bond dissociation enthalpies (BDEs) given for C_α−H and the hydrogen atoms in the CH_3 side groups of $MeNH-CHMe-CO_2^-$, an amino acid not experimentally investigated in this study; see Scheme 3. For C_α−H in $MeNH-CHMe-CO_2^-$, the BDE may well be a few kJ mol^{-1} weaker than that in Gly^-.[30] However, the general weakness of the C_α−H bond (360 kJ mol^{-1}) relative to all other C−H and N−H bonds is quite evident. Attacking $H^{\bullet}$ atoms

SCHEME 3: H Abstraction Sites and BDEs of C−H/N−H/O−H Bonds at 300 K in kJ/mol (in parentheses): Numbers in Bold Denote Radicals Derived from H Abstraction



[a] BDE for a secondary amine from ref 27. Value for Gly^- is 403 from ref 26. [b] From ref 26 for Gly−. [c] From ref 28 for a secondary amine. [d] From ref 29 for isobutane. [e] From ref 29.

should, therefore, indeed preferentially undergo reaction 22, and give the radical **2**, $NR_2-{}^{\bullet}CR-CO_2^-$, as the major product.

To test for this reaction experimentally, an N_2O-saturated aqueous solution containing 2 M 2-methyl-2-propanol and 0.1 M Gly at pH 9.05 ($[Gly^{\pm}] = 78$ mM, $[Gly^-] = 22$ mM) was pulse irradiated. It led to the formation of an optical absorption at 250−300 nm with biphasic kinetics as shown in Figure 1a. The primary water radicals in this solution, ${}^{\bullet}OH$ ($G \sim 0.7\ \mu M\ J^{-1}$) and $H^{\bullet}$ ($G \sim 0.06\ \mu M\ J^{-1}$), react with 2-methyl-2-propanol with rate constants of $k_{23} = 5 \times 10^8\ M^{-1}\ s^{-1}$ and $k_{24} = 1.7 \times 10^5\ M^{-1}\ s^{-1}$, respectively.[20] Considering the $k({}^{\bullet}OH,Gly^{\pm})$ and $k({}^{\bullet}OH,Gly^-)$ rate constants from Table 2 and the respective concentrations, it is evident that practically all hydroxyl radicals will have reacted with 2-methyl-2-propanol (reaction 23) and that the first fast increase of absorbance should belong to ${}^{\bullet}CH_2C(CH_3)_2OH$ radicals.

$$ {}^{\bullet}OH + (CH_3)_3COH \rightarrow {}^{\bullet}CH_2C(CH_3)_2OH + H_2O \quad (23)$$

$$ H^{\bullet} + (CH_3)_3COH \rightarrow {}^{\bullet}CH_2C(CH_3)_2OH + H_2 \quad (24)$$

Indeed, taking $\epsilon \sim 200\ M^{-1}\ cm^{-1}$ for this radical at 260 nm,[31] its yield was calculated to $\sim 0.8\ \mu M\ J^{-1}$, which is as expected for the full conversion of hydroxyl radicals into ${}^{\bullet}CH_2C(CH_3)_2OH$. The second slower part of the kinetic curve ($t_{1/2} \sim 0.2\ \mu s$) cannot, however, be explained by the additional formation of the same ${}^{\bullet}CH_2C(CH_3)_2OH$ radical in the reaction of 2-methyl-2-propanol with H atoms, eq 24. First of all, the kinetics depend on the glycine anion concentration and not on that of the 2-methyl-2-propanol as in the initial fast step. In addition, the total absorbances observed for the two processes are approximately equal and, under this scenario, thus are in conflict with the ~10:1 yield ratio of radiolytically produced ${}^{\bullet}OH$ and $H^{\bullet}$. The secondary process is, therefore, ascribed to the formation of $NH_2-{}^{\bullet}CH-CO_2^-$ radicals, which are known to absorb in the UV region[15] with albeit unknown extinction coefficients. A plot of k_{obs} for this growth vs $[Gly^-]$ is shown in Figure 1b, and from the slope a second-order rate constant of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ was calculated.

Two reactions must be considered for production of this radical by an H atom abstraction from $C_{\alpha}-H$ of Gly^-: (i) reaction 25 involving ${}^{\bullet}CH_2C(CH_3)_2OH$ radicals (formed by reactions 23 and 24) or reaction 22 involving $H^{\bullet}$ atoms.

$$ {}^{\bullet}CH_2C(CH_3)_2OH + NH_2-CH_2-CO_2^- \rightarrow NH_2-{}^{\bullet}CH-CO_2^- + C(CH_3)_3OH \quad (25)$$

Since these radicals would be present in the above-mentioned 10:1 concentration and the measured rate constant is high enough to ensure complete scavenging by Gly^- under the experimental conditions in either case, quantitative determination of the $NH_2-{}^{\bullet}CH-CO_2^-$ yield should reveal which is the relevant process for its formation. To find out, the yield of the presumed $NH_2-{}^{\bullet}CH-CO_2^-$ radicals was probed by redox titration with MV^{2+} (reaction 26). This process yields $MV^{\bullet+}$

$$ NH_2-{}^{\bullet}CH-CO_2^- + MV^{2+} \rightarrow {}^{+}NH_2{=}CH-CO_2^- + MV^{\bullet+} \quad (26)$$

radical cations which are easily detectable by their intense absorbance at 600 nm (Table 1). The ${}^{\bullet}CH_2C(CH_3)_2OH$ radical, on the other hand, is inert toward MV^{2+}. In addition, when $k_{26}[MV^{2+}] \gg k_{22or25}[Gly^-]$ holds, the formation of $NH_2-{}^{\bullet}CH-CO_2^-$ becomes the rate-determining step for the growth kinetics of the $MV^{\bullet+}$ radical, providing an independent possibility to evaluate the kinetics of the H abstraction from Gly^-.

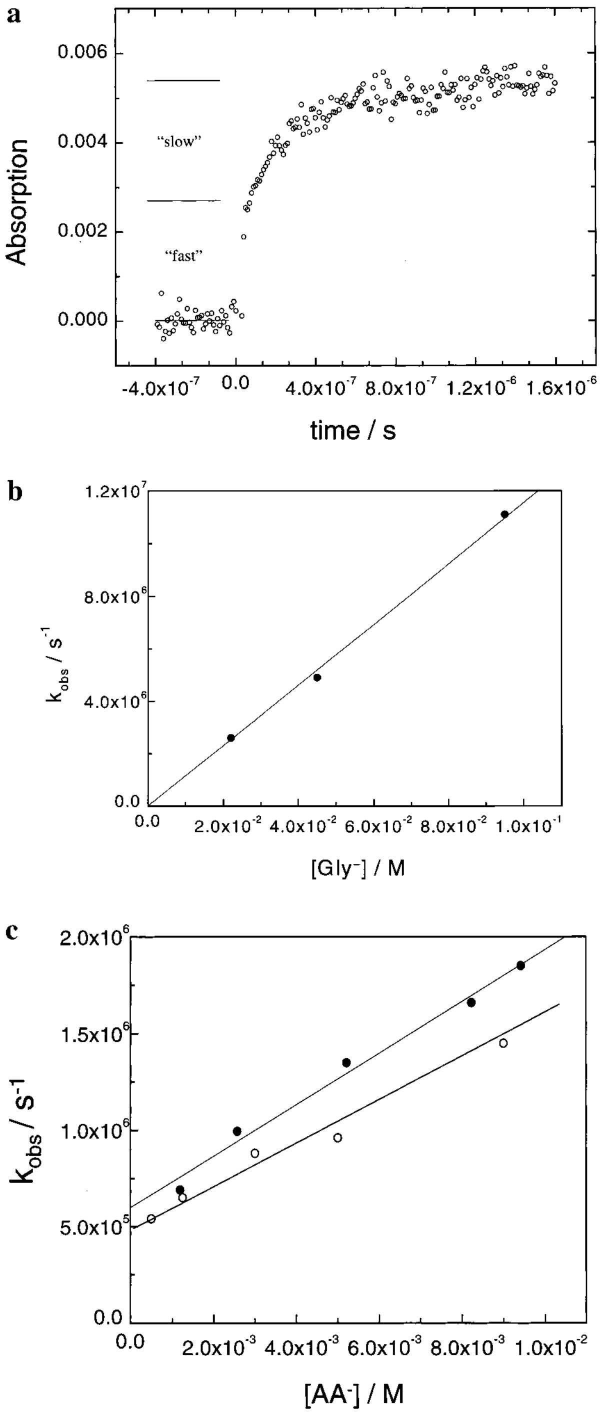


Figure 1. (a) Optical absorption at 260 nm vs time trace, recorded upon pulse irradiation of a deoxygenated, N_2O-saturated aqueous solution containing 2 M 2-methyl-2-propanol and 0.1 M glycine, pH 9.55. (b) Plot of k_{obs} (pseudo-first-order formation rate constants at 260 nm) for the slow process shown in a) vs $[Gly^-]$ in the same solution at different pH in the range of 9−11. (c) Plot of k_{obs} (pseudo-first-order $MV^{\bullet+}$ formation rate constants at 600 nm) vs $[AA^-]$ determined in deoxygenated, N_2O-saturated aqueous solutions containing 2 M 2-methyl-2-propanol, 0.5 mM MV^{2+} and 10 mM Gly (○), or *N,N*-Me_2Gly (●) at different pH in the range of 9−11 to change $[AA^-]$.

Concerning experimental verification it must be recognized that the MV^{2+} reduction generally exhibited two distinct processes with, however, only one of them being relevant to the aspect under discussion. For example, when 0.5 mM MV^{2+} was added to these systems, a fast initial step, accounting for the $MV^{\bullet+}$ formation at a yield of $G \sim 0.05\ \mu M\ J^{-1}$, can unambiguously be attributed to the reduction of MV^{2+} by hydrated electrons in a competition to the e_{aq}^- reaction with N_2O (for the respective rate constants see ref 20). The reaction of interest associated with the glycine-derived reducing radical is the slower second process. It results in similar low yields ($G \sim 0.05 \pm 0.02\ \mu M\ J^{-1}$), clearly pointing at reaction 22 as being responsible for this process. Also, since the rate of $MV^{\bullet+}$ formation was pseudo-first order in [Gly^-] and remained independent of [MV^{2+}], the condition $k_{26}[MV^{2+}] \gg k_{22}[Gly^-]$ held. Thus, k_{22} could be determined from the slope of the plot of k_{obs} for the absorption growth vs [Gly^-], shown in Figure 1c. The straight line gave $k_{22,Gly^-} = (1.0 \pm 0.1) \times 10^8\ M^{-1}\ s^{-1}$. Within this picture, the intercept at [Gly^-] = 0 would reflect the competing reactions of H atoms with 2-methyl-2-propanol, eq 24, and with MV^{2+} ($k = 6 \times 10^8\ M^{-1}\ s^{-1}$).[20] The estimated combined rate for H atom removal by these two processes is $\sim 6 \times 10^5\ s^{-1}$. Its good agreement with the experimental intercept value of $k_{obs} = (4.5 \pm 0.4) \times 10^5\ s^{-1}$ thus supports our assignment.

When *N*,*N*-Me_2Gly was used as reactant instead of Gly in these experiments with MV^{2+}, very similar values were obtained; k_{22} as derived from Figure 1c was $(1.4 \pm 0.1) \times 10^8\ M^{-1}\ s^{-1}$ and the intercept was $(5.7 \pm 0.3) \times 10^5\ s^{-1}$. However, when MeAla was used (with concentrations of the anionic form up to 9 mM) no significant acceleration of the secondary $MV^{\bullet+}$ formation rate above $k_{obs} \sim 6 \times 10^5\ s^{-1}$ was observed. This leaves an upper limit of $<10^7\ M^{-1}\ s^{-1}$ for the H atom reaction with $MeAla^-$. As this amino acid has no H atom on the activated C_α position, that result is entirely reasonable. It also indicates that H atom abstractions from the side chain CH_3 group and the N−H bonds of the amino group by $H^\bullet$ radicals occur with at least 1 order of magnitude slower rates than H abstractions from the α-carbon. (One should note that the formation of radicals of types **1** and **4** would not give enhanced $MV^{\bullet+}$ absorbance on the time scale of Figure 1c, but their production would have caused the rate of H atom removal to rise, and therefore, k_{obs} would have increased with [$MeAla^-$].)

Measurements were also made for the amino acid glycine in solutions containing 0.2 M dimethyl sulfoxide (DMSO) instead of 2-methyl-2-propanol as $^\bullet OH$ radical scavenger. In this system all $^\bullet OH$ radicals were converted into methyl radicals, eq 27. $H^\bullet$ atoms, on the other hand, could react with DMSO ($k \sim 3 \times 10^6\ M^{-1}\ s^{-1}$)[20] and MV^{2+}, but also at increased probability with Gly^- as the concentration of the latter was raised.

$$^\bullet OH + (CH_3)_2SO \rightarrow {}^\bullet CH_3 + CH_3SO_2H \tag{27}$$

The results were very similar to those obtained with the 2-methyl-2-propanol system. The yield of a slow-growth section of $MV^{\bullet+}$ formation was again only of the order of $0.05\ \mu M\ J^{-1}$, concurring with the H atom yield. From the slope of k_{obs} for this secondary growth vs [Gly^-] $k_{22,Gly^-} = (1.7 \pm 0.2) \times 10^8\ M^{-1}\ s^{-1}$ was obtained. The [Gly^-]-independent intercept amounted to $(1.0 \pm 0.1) \times 10^6\ s^{-1}$, in very good agreement with $0.9 \times 10^6\ s^{-1}$ calculated for the combined rate of $H^\bullet$ removal by MV^{2+} and DMSO.

The k_{22,Gly^-} values evaluated from the MV^{2+} experiments with the two $^\bullet OH$ radical scavengers 2-methyl-2-propanol and dimethyl sulfoxide are close to that of $1.2 \times 10^8\ M^{-1}\ s^{-1}$ obtained directly from the growth of the secondary optical absorption at 250−300 nm in the absence of MV^{2+} (Figure 1b). Hence, all of the present data are consistent with an average $k_{22,Gly^-} = (1.4 \pm 0.4) \times 10^8\ M^{-1}\ s^{-1}$. The finding of a practically identical value for *N*,*N*-Me_2Gly^- $(1.4 \pm 0.1) \times 10^8\ M^{-1}\ s^{-1}$) makes sense, since the N-attached methyl groups should not significantly affect the H abstraction at the C_α site. The overall significant point is that H atom reactions with the anions of the AAs are about a factor of 10 slower than those of the hydroxyl radicals. This is consistent with the lower exothermicity for the formation of H_2 than H_2O in the H abstraction (cf. the BDEs of H_2 and H_2O in Scheme 3).

In conclusion, whenever H atoms are present on the C_α position, this is the main site of H atom attack. These reactions, occurring with rate constants on the order of $10^8\ M^{-1}\ s^{-1}$, are sufficiently fast to contribute quantitatively to the yields of α-amino-α-carboxyl radicals **2**. They must, accordingly, be taken into account, even on the pulse radiolysis time scale. From the results with MeAla, on the other hand, one can conclude that H atom abstractions from the N−H bonds of amino groups or from the CH_3 side chains in substituted glycines are at least 1 order of magnitude slower than from the C_α position. Consequently, these latter reactions of $H^\bullet$ (but only these) can be neglected for all amino acids containing C_α−H bonds.

A further conclusion concerns H abstraction from C_α−H by $^\bullet CH_2C(CH_3)_2OH$ and $^\bullet CH_3$ (reaction 25 and analogous one), which are also presumed to yield predominantly radical **2**. These reactions must, however, proceed with rate constants well below $10^6\ M^{-1}\ s^{-1}$. If instead this or an even higher rate applied, the reduction of MV^{2+} by the thus formed **2** radical would exhibit exponential kinetics with $t_{1/2} \sim 100\ \mu s$ under the experimental conditions (10 mM AA^- and 0.5 mM MV^{2+}). But no such process was observed at this time scale. Also, as pointed out above, the yield would be much larger than $0.05\ \mu M\ J^{-1}$. This finding is consistent with a reported rate constant of only 120 $M^{-1}\ s^{-1}$ for the reaction of $^\bullet CH_3$ with Gly^- [32] and similarly low values for H abstraction from ethanol and 2-propanol, i.e., from compounds with comparable C−H bond dissociation energies.

Finally, the reactions of $H^\bullet$ with the anions of the AAs are much faster than those with the zwitter ions for basically the same reasons as discussed for the $^\bullet OH$-induced reactions.

3. Yields of Radicals from $^\bullet$OH-Induced Reactions. A further objective of this study was to identify the different primary radicals formed by $^\bullet OH$ attack on the various amino acid anions and to determine their yields in relation to that of the initiating $^\bullet OH$. It must be recognized that some of the radical types shown in Schemes 1 and 2 are not possible with certain specific structures. The exclusions are: **1** and **5** in the case of glycine, **5** for alanine, **2** and **5** for α-methylalanine, and **1** and **4** for *N*,*N*-dimethylglycine.

As explained in the Introduction section, the yields of primary amino acid radicals were obtained by following their reactions with selective redox scavengers. Radicals **2**, **5**, and **6** are C_α-centered and, as the result of the activating α-amino group, inherently reducing in character. Radical **1** is also reducing but to a much lesser extent. Radicals **3** and **4**, on the other hand, are expected to be oxidants as most N-centered radicals. However, because of its extremely short lifetime, species **3** cannot be scavenged directly. Determination of its yields is only possible through titration of its decay product, the strongly reducing species **6**. The observed reactivities of each radical type toward the various redox scavengers are summarized in Table 1. If the rate was equal or close to the diffusion limit (and thus assured complete scavenging), a "+" is given. For $^\bullet CO_2^-$ numerical values are listed since they are independent of which amino acid is used. For cases where scavenging is slower than diffusion-controlled, but fast enough to be used for the pulse radiolysis measurements, the range of numerical values

TABLE 3: Absolute (in Units of μmol J^{-1}) and Relative Yields of Reducing Radicals Produced in Pulse-Irradiated N_2O-Saturated Aqueous Solutions of *N*,*N*-Me_2Gly in the Presence of CB^- or MV^{2+} as Scavengers

Scavenger: CB^-

[*N*,*N*-Me_2Gly] mM	pH	$G(^{\bullet}OH)_{abs}$[a]	$G(^{\bullet}OH + H^{\bullet})_{abs}$[b]	$G(^{\bullet}CB^{2-})_{abs}$	$G(\mathbf{6})_{rel}$[c] $[G(^{\bullet}CB^{2-})_{abs}/G(^{\bullet}OH)_{abs}]$
50	10.9	0.656	0.716	0.53	0.81
100	10.7	0.68	0.74	0.56	0.82
450	10.7	0.74	0.80	0.57	0.77
av					**0.80 ± 0.02**

Scavenger: MV^{2+}

[*N*,*N*-Me_2Gly] mM	pH	$G(^{\bullet}OH)_{abs}$[a]	$G(^{\bullet}OH + H^{\bullet})_{abs}$[b]	$G(MV^{\bullet +})_{abs}$	$G(\mathbf{2})_{abs}$[d]	$G(\mathbf{2})_{rel}$ $[G(\mathbf{2})_{abs}/G(^{\bullet}OH)_{abs}]$	$G(MV^{2+}$ reduction$)_{rel}$ $[G(MV^{\bullet +})_{abs}/G(^{\bullet}OH + H^{\bullet})_{abs}]$
0.8	11.0	0.56	0.62	0.624	0.116	0.21	1.01
5	9.7	0.575	0.635	0.655	0.135	0.23	1.03
5	11.0	0.59	0.65	0.636	0.104	0.18	0.98
100	10.6	0.68	0.74	0.737	0.133	0.20	1.00
av						**0.21 ± 0.02**	**1.01 ± 0.02**

[a] Calculated by using formula I. [b] $G(H^{\bullet}) = 0.06$ μmol J^{-1} for all amino acid concentrations. [c] May include some radical **5**. [d] Calculated by using formula II.

is given, while a “−” symbol is shown, if the rate was negligible or the reaction was not possible.

*Yields of Radicals **2**, **5**, and **6**.* α-Amino radicals of type **5** and **6** are characterized by large negative reduction potentials for the half reaction 28[33] and, accordingly, found to be good reductants.[34]

$$^{+}NR{=}CR_2 + e^- \rightarrow NR_2\text{-}^{\bullet}CR_2 \qquad (28)$$

Specifically, the reduction potential of $^{\bullet}CH_2{-}NH_2$ (i.e., radical **6** from glycine) has been estimated to be −1.5 V.[35] Radicals **6** reduce, for example, the anion of 4-carboxybenzophenone (CB^-) with rate constants of ≥ 3 × 10^8 M^{-1} s^{-1}.[3] The corresponding rate of MV^{2+} reduction should be faster, due to the less negative value of $E°$ (−0.45 V for $MV^{2+}/MV^{\bullet +}$ vs −1.13 V for $CB^-/^{\bullet}CB^{2-}$) (see Table 1). Discrimination between $^{\bullet}CH_2{-}N(CH_3){-}CH_2{-}CO_2^-$ (radical **5**) and $(CH_3)_2N{-}^{\bullet}CH_2$ (radical **6**), both of which are likely to be formed from *N*,*N*-Me_2Gly, is not expected because their reduction potentials should be very similar. In this case only a careful end-product analysis may eventually allow quantification of their yields. However, no attempt in this direction was made in the present work.

Because the radical site in **2** is alpha to *both* the amino *and* the carboxy*l* group this type of radical gains extra resonance stabilization through the captodative effect.[26] It is, therefore, more stabilized than radicals **5** and **6**. Consequently its reduction potential should be less negative than −1.5 V, and **2** should, consequently, be less prone to oxidation. In fact, the reduction potential of **2** in acetonitrile was found to be about 1 V more positive than for radicals **5** and **6**.[36] The evidence presented below, namely, that **2** was able to reduce MV^{2+} but not CB^-, places its reduction potential in aqueous solution into the −1.13 to −0.45 V range. Applying these two electron acceptors thus allowed unambiguous distinction between **2**, on one hand, and **5** + **6**, on the other hand.

The yields for *N*,*N*-Me_2Gly will be considered first, since the relatively long-lived oxidizing ($-N^{\bullet}-$) aminyl radical **4** cannot be produced from this compound and this simplifies the interpretation of the results. According to the primary reactions of $^{\bullet}OH$ in Scheme 1, the pulse radiolysis of N_2O-saturated aqueous solutions of this AA^- at pH 10−11 produces **2**, **5**, and **6** as reducing radicals (reactions 3, 4 followed by 9 and/or 7a or b, and 6). Further yields of radical **2** result from H atom reaction (reaction 22). Titration of the reducing radicals by methyl viologen gave an overall second-order rate constant of 4.2 × 10^9 M^{-1} s^{-1} for the MV^{2+} reduction. When CB^- was used as the scavenger, its reduced form $^{\bullet}CB^{2-}$ was produced in the combined reactions of **5** and **6** with a second-order rate constant of 1.5 × 10^9 M^{-1} s^{-1}.

The absolute yields of $MV^{\bullet +}$ and $^{\bullet}CB^{2-}$ obtained at different *N*,*N*-Me_2Gly^- concentrations are shown in Table 3 in the columns denoted as $G(MV^{\bullet +})_{abs}$ and $G(^{\bullet}CB^{2-})_{abs}$, respectively. Both increase slightly with [*N*,*N*-Me_2Gly^-], reflecting the increased scavenging efficiency of $^{\bullet}OH$ radicals according to eq I. $G(MV^{\bullet +})_{abs}$, specifically, is always equal to the sum of $^{\bullet}OH$ and $H^{\bullet}$ radicals, with $G(MV^{\bullet +})_{rel} = G(MV^{\bullet +})_{abs}/G(^{\bullet}OH + H^{\bullet}) = 1.01 \pm 0.02$ (Table 3 last column). This means that MV^{2+} must have scavenged all radicals formed upon reactions of $^{\bullet}OH$ and $H^{\bullet}$ with $Me_2N{-}CH_2{-}CO_2^-$. The yields of $^{\bullet}CB^{2-}$ were also high, albeit systematically lower than those of $MV^{\bullet +}$ measured under similar conditions. The obvious candidate for this missing fraction is radical **2**. The yield of $^{\bullet}CB^{2-}$, consequently, results exclusively from the α-amino radicals **5** and **6**, with the decarboxylated radical **6** assumed to be the major component.

Since H atoms contribute only to the yield of **2** (see section **2**) the yield of CB^--reducing α-amino radicals is reasonably related only to the yield of $^{\bullet}OH$. Indeed, $G(^{\bullet}CB^{2-})_{abs}/G(^{\bullet}OH)$ has a constant value which numerically amounts to 0.80 ± 0.02. Although this value can be assumed to reflect primarily the yield of **6** it should be recalled that it may include some radical **5** formed by the reactions of $^{\bullet}OH$ (reactions 6 and/or 4 followed by 7b, Scheme 1).

The absolute yield of the α-amino-α-carboxyalkyl radical **2** is, as mentioned already, due to reactions of both $^{\bullet}OH$ and $H^{\bullet}$. Subtracting the $G = 0.06$ mM J^{-1} contribution by H atoms the absolute yield of **2** formed in the reaction of $^{\bullet}OH$ can be calculated via eq II. From this, $G(\mathbf{2})_{rel} = G(\mathbf{2})_{abs}/G(^{\bullet}OH) = 0.21 \pm 0.02$. All these data are listed in Table 3.

$$G(\mathbf{2})_{abs} = G(MV^{\bullet +})_{abs} - G(H^{\bullet}) - G(^{\bullet}CB^{2-})_{rel}G(^{\bullet}OH) \qquad (II)$$

The procedure for obtaining the radical yields for the amino acids other than *N*,*N*-Me_2Gly had to be modified to allow for the presence of $RN^{\bullet}{-}CR_2{-}CO_2^-$ aminyl radicals. Because of the transformation of these oxidizing radicals into the reducing **2**, **6,** and **7** radicals (Scheme 2) these systems exhibit a secondary comparatively slow reduction step of MV^{2+} and CB^- [1,2] in addition to the reduction by the primary, directly formed **2**, **6**, and **5** radicals described above for the *N*,*N*-Me_2Gly exemplary system. Experimentally the two reduction steps could always clearly be separated. Under our experimental conditions the faster process typically occurred in the nanosecond to lower

TABLE 4: Relative Yields of Radical Formed in Reactions of Amino Acid Anions with $^{\bullet}$OH

amino acid anion	$G(\mathbf{6})_{rel}$ α-aminoalkyl radical	$G(\mathbf{2})_{rel}$ α-amino-α-carboxy radical	$G(\mathbf{4})_{rel}$ aminyl radical	$G(\mathbf{1})_{rel}$ side-chain radical
Gly$^-$	0.22 ± 0.02	0.37 ± 0.04	0.36 ± 0.05	
Ala$^-$	0.25 ± 0.02	0.22 ± 0.03	0.47 ± 0.05	0.05 ± 0.05
MeAla$^-$	0.23 ± 0.02[a] 0.27 ± 0.01[b] (av 0.25 ± 0.02)		0.61 ± 0.04	0.18 ± 0.04
N,N-Me$_2$Gly$^-$	0.80 ± 0.02[c]	0.21 ± 0.02		

[a] Determined from "fast" $G(\mathrm{MV}^{\bullet+})$ contribution; ref 2. [b] Determined from "fast" $G(^{\bullet}\mathrm{CB}^{2-})$ contribution; ref 2. [c] May include some radical **5**.

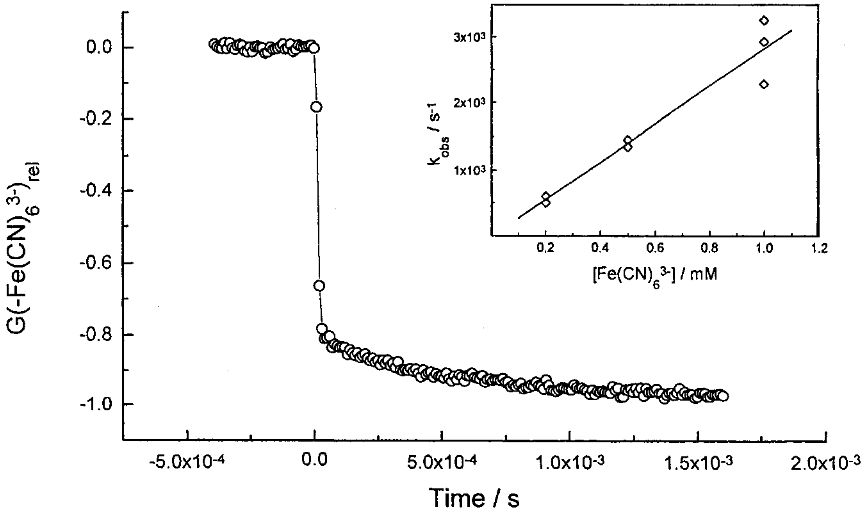


Figure 2. Optical absorption trace at 420 nm (presented as a relative yield of bleached $\mathrm{Fe(CN)_6^{3-}}$) vs time recorded in pulse irradiated, deoxygenated, N_2O-saturated aqueous solution containing 0.1 M MeAla and 0.5 mM $\mathrm{Fe(CN)_6^{3-}}$ at pH 10.0. Inset*:* Plot of k_{obs} for the slow bleaching process in the same solution vs $[\mathrm{Fe(CN)_6^{3-}}]$.

microsecond range, while the secondary slower one proceeded on the tens of microsecond time scale. Furthermore, in the methyl viologen containing systems the rate for the secondary reduction does not depend on the MV^{2+} concentration, i.e., the rate-determining step is the transformation of radical **4** into the reducing species. Accordingly, the initial yields of radicals **2**, **5**, and **6** are determined from the fast or primary $\mathrm{MV}^{\bullet+}$ and $^{\bullet}\mathrm{CB}^{2-}$ yields. They are summarized in Table 4.

Yields of Aminyl Radicals 4. For all systems where aminyl radicals **4** can be formed, their absolute yield is given by that of $\mathrm{MV}^{\bullet+}$ from the slower secondary growth, i.e., $G(\mathbf{4})_{abs} = G(\mathrm{MV}^{\bullet+})_{sec}$. Thus, the yield of aminyl radicals relative to $^{\bullet}$OH amounts to $G(\mathbf{4})_{rel} = G(\mathrm{MV}^{\bullet+})_{sec}/G(^{\bullet}\mathrm{OH})$. The other procedures for identification and quantification of **4**, namely, oxidation of hydroquinone by this radical, and evidence for $^{\bullet}\mathrm{CO_2}^-$ as a result of β elimination (reaction 12) have already been presented.[2] In the present study only the total yield of radical **4** and not its secondary partition into **2**, **6,** and **7** (Scheme 2) was of concern. The explicit values of $G(\mathbf{4})$ for Gly$^-$, Ala$^-$, and MeAla$^-$ have been summarized in Table 4, column four.

Yields of Side-Chain Radicals. The formation of side-chain radical **1** is only possible in the reactions of $^{\bullet}$OH with Ala and MeAla anions. Like the β-hydroxyalkyl radical $^{\bullet}\mathrm{CH_2C(CH_3)_2}$-OH derived from 2-methyl-2-propanol, **1** is also relatively inert toward redox processes. Because it is the only C-centered radical that cannot reduce MV^{2+}, its yield may thus be obtained from the difference between the calculated $G(^{\bullet}\mathrm{OH} + \mathrm{H}^{\bullet})$ and the experimentally determined total $\mathrm{MV}^{\bullet+}$ yield. Direct identification and quantitative determination of radicals **1** can, however, be achieved by using $\mathrm{Fe(CN)_6^{3-}}$ as a specific scavenger. With $E°(\mathrm{Fe(CN)_6^{3-}/Fe(CN)_6^{4-}}) = +0.36$ V, this scavenger is able to accept electrons even from alkyl radicals lacking activation by α-positioned functional groups.[37] Figure 2 shows an optical absorption trace taken at 420 nm in N_2O-saturated, pH 10.0, aqueous solution containing 0.1 M MeAla and 0.5 mM $\mathrm{Fe(CN)_6^{3-}}$. The intensity on the *y*-axis is scaled to the yield of bleached $\mathrm{Fe(CN)_6^{3-}}$ relative to the yield of $^{\bullet}$OH radicals and denoted as $G(-\mathrm{Fe(CN)_6^{3-}})_{rel}$. This was obtained by normalizing the signal for dosimetry, the known extinction coefficient of $\mathrm{Fe(CN)_6^{3-}}$ at 420 nm, and $G(^{\bullet}\mathrm{OH})$. It can be seen that the radicals formed from MeAla$^-$ reduced $\mathrm{Fe(CN)_6^{3-}}$ practically quantitatively ($G(-\mathrm{Fe(CN)_6^{3-}})_{rel} \cong 0.98$) on the full 2 ms time scale. However, this occurs in two well separated processes. The first, fast one accounts for ~80% decrease in absorbance. This is followed by a second, much slower process, amounting to ~18% of the overall yield. The yield of the first step agrees reasonably well with the relative total yield of reduced MV^{2+}, corresponding to all radicals formed in reactions 4 followed by 9, or 8 and/or 5 followed by 12 or 13 + 9, which are not kinetically separated on this 2 ms time scale.[2] The yield of the slow process in Figure 2 fits reasonably well the expected yield of C-centered side-chain radical **1** calculated from $G(^{\bullet}\mathrm{OH} + \mathrm{H}^{\bullet}) - G(\mathrm{MV}^{\bullet+})_{total}$.

The pseudo-first-order kinetics of this slow process was linearly dependent on the $\mathrm{Fe(CN)_6^{3-}}$ concentration as shown in the insert of Figure 2. From the slope of the straight line a rate constant of $k_{29} = (2.8 \pm 0.4) \times 10^6\ \mathrm{M^{-1}\ s^{-1}}$ has been obtained. This is the same order of magnitude as the known values for the reaction of $\mathrm{Fe(CN)_6^{3-}}$ with other alkyl radicals such as $^{\bullet}\mathrm{CH_3}$ or $^{\bullet}\mathrm{CH_2CO_2H}$.[37]

$$\mathrm{NH_2{-}(CH_3)C(^{\bullet}CH_2)\text{-}CO_2^{\,-} + Fe(CN)_6^{\,3-} \rightarrow products} \quad (29)$$

Summary of Relative Yields for $^{\bullet}$OH Reactions. Table 4 summarizes all the relative yields of the various primary radicals from Gly$^-$, Ala$^-$, MeAla$^-$, and *N,N*-Me$_2$Gly$^-$ as the result of

TABLE 5: Total and Partial Rate Constants for the Attack of $^{\bullet}$OH Radicals on Amino Acid Anions (in Units of 10^9 M^{-1} s^{-1}) Yielding the Radicals Denoted with Bold Numbers

amino acid anion	k(total)	k(**2**)	k(N-attack) [k(**6**) + k(**4**)]	k(**6**)	k(**4**)
Gly^-	(2.8 ± 0.1)	(1.04 ± 0.12)	(1.63 ± 0.15)	(0.62 ± 0.06)	(1.01 ± 0.14)
Ala^-	$(1.74 \pm 0.02)^a$	(0.38 ± 0.05)	(1.25 ± 0.09)	(0.44 ± 0.04)	(0.82 ± 0.09)
$MeAla^-$	$(2.4 \pm 0.1)^b$		(2.06 ± 0.12)	(0.60 ± 0.05)	(1.46 ± 0.11)
N,N-Me_2Gly^-	(3.6 ± 0.2)	(0.76 ± 0.08)	(2.88 ± 0.18)	$(2.88 \pm 0.18)^c$	

[a] Includes H abstraction from side-chain C−H (≈5%). [b] Includes H abstraction from side-chain C−H (18%). [c] Includes unknown (probably minor) contribution of H abstraction from N−Me groups.

their reaction with $^{\bullet}$OH. In addition to the points discussed earlier, some further general comments are deemed to be of interest.

In particular, we would like to draw attention to the relative yields of α-aminoalkyl and α-amino-α-carboxyalkyl radicals produced in the direct reaction of $^{\bullet}$OH radicals with glycine anions, i.e., $^{\bullet}CH_2-NH_2$ and $NH_2-{}^{\bullet}CH-CO_2^-$. The results obtained in this study allow us to correct an earlier erroneous assignment,[1] namely, that the decarboxylated $^{\bullet}CH_2-NH_2$ radical was the only reducing species reacting with methyl viologen. The unambiguous distinction between $^{\bullet}CH_2-NH_2$ and $NH_2-{}^{\bullet}CH-CO_2^-$, and quantification of their yields by using CB^- as a scavenger have revealed that, indeed, both are generated already in the primary reaction of $^{\bullet}$OH. The formation of $NH_2-{}^{\bullet}CH-CO_2^-$ is, therefore, not only due to secondary reactions as presumed earlier. In addition, the present investigation showed that a significant fraction of these α-amino-α-carboxyalkyl radicals originates from H atom reaction. Furthermore, it was gratifying to see that ESR, concurrent with our optical titration experiments, has now also been successful in identifying $^{\bullet}CH_2-NH_2$, $^{\bullet}NH-CH_2-CO_2^-$ and $CO_2^{\bullet-}$,[11−13,38] and thus substantiating the mechanism based on the pulse experiments and outlined in Schemes 1 and 2.

For comparison of relative yields obtained by the two experimental approaches, the fractions of α-amino-α-carboxyalkyl and α-aminoalkyl radicals formed by the combined reactions of $^{\bullet}$H and $^{\bullet}$OH with glycine are 0.45 (**2**) and 0.21 (**6**) from the present optical titration, and 0.53 (**2**) and 0.29 (**6**) from the ESR study. Although they do not match exactly, the agreement is, nevertheless, reasonable in view of the difficulties involved in quantitative yield evaluations from the time-resolved ESR data. Also, the ESR method does not yet include a total radical balance, with a quantitative measure of the aminyl radical yields still pending.

With respect to another detail, it should be noted that some of the Ala^- and $MeAla^-$ radical yields are based on absolute yields measured in reference.[2] However, the relative yields in Table 4 differ slightly from those reported there due to a difference in $G(^{\bullet}OH)$ applied. The latter has been recalculated for this work by using the newly determined values for $k(^{\bullet}OH+AA^-)$ in Table 2.

4. Rate Constants for Formation of Specific Radicals by Reactions of $^{\bullet}$OH. Table 5 summarizes the overall rate constants k(total) for the reaction of $^{\bullet}$OH radicals with Gly^-, Ala^-, $MeAla^-$, and N,N-Me_2-Gly^-. Displayed are also the partial rate constants for the formation of specific radicals generated by the reactions in Scheme 1. These rate constants were obtained by multiplying k(total) by the relative yields of the respective radicals, listed in Table 4 for each AA^-.

The overall rate constants are all of the same order of magnitude, between $(1.5-4) \times 10^9$ M^{-1} s^{-1}. Direct comparison and evaluation of the observed differences are, however, not meaningful since k(total) reflects various individual reactions. Furthermore, some of them do not occur for certain amino acids. Our discussion is, therefore, confined to the partial rate constants describing the various individual processes.

Formation of the side-chain radicals (**1**) is least favored. The partial rate constant for its formation from $MeAla^-$ is $\sim 4 \times 10^8$ M^{-1} s^{-1}. This amounts to about 7×10^7 M^{-1} s^{-1} per H atom which is about one-seventh the rate for H atom abstraction from the C_α in glycine. For alanine, the low yield of radical **1** prevents any rate evaluation within reasonable error limits. Relatively low rate constants for side-chain abstractions are, however, in any case fully corroborated by the BDEs in Scheme 3, i.e., on the ground of energetic considerations. Abstraction reactions from side chains do, however, play a more significant role in larger amino acids and peptides, and at lower, near neutral pH.[21,39]

Concerning the H abstraction from $C_\alpha-H$ it is noted that k(**2**) is almost three times higher for glycine than for alanine although the number of $C_\alpha-H$ bond differs only by a factor of 2. Interestingly, an exact statistical 2:1 ratio is found for the respective k(**2**) values for N,N-Me_2Gly^- and Ala^-. We like to argue that the relatively higher value for the glycine anion reflects the extraordinary low $C_\alpha-H$ bond energy and a minimum of steric hindrance for the H abstraction, as well as an exceptionally favorable capto-dative resonance stabilization of the radical **2** resulting from this process. It must, however, be kept in mind that the titration of **2** does not only cover the yield of this radical formed directly via H abstraction but may also include a contribution via the reaction sequence 1, 4, and 7a. The rate constants k(**2**) may thus only represent an upper limit for reaction 3.

An important feature, particularly in the light of previous controversial discussion, is the now unambiguously established fact, that direct attack at nitrogen is the dominant reaction of the hydroxyl radical. This clearly emerges from the combined k(**6**) + k(**4**) values, listed as k(N-attack), in comparison with k(**2**). For those three amino acid anions which carry $C_\alpha-H$ bonds, namely Gly^-, Ala^-, and N,N-Me_2Gly^-, the ratio between k(N-attack) and k(**2**) per $C_\alpha-H$ bond amounts to 3.1, 3.3, and 7.6, respectively. The especially high value for N,N-Me_2Gly^- plausibly finds an explanation in the electron-inductive effect of the two methyl groups attached directly to the nitrogen atom which would lower the ionization potential of the amino function. In line with this argument, $k(\text{total}) = 3.1 \times 10^9$ M^{-1} s^{-1} for sarcosine (N-methylglycine)[21] is about halfway between that for N,N-Me_2Gly^- and Gly^-. Even more striking support can be drawn from the partial rate constants k(**6**) for the decarboxylation route (reaction sequence 1, 4 and 9). Here, the N,N-Me_2Gly^- value is about 5 times higher than the values for the other three amino acids which carry only hydrogen at the amino function.

The partial rate constants k(**6**) are almost the same for Gly^-, Ala^-, and $MeAla^-$. In fact, one would expect little if any variation for this value since electron density at the nitrogen atom should not be affected significantly by C_α substitution. This pertains to both, the initial $^{\bullet}$OH attack at nitrogen as well as to the decarboxylation of the caged radical zwitterion **3**. Another significant parameter, namely, steric accessibility of N, may decrease a bit within this series providing a rationale for the slightly lower value for alanine. The fact that the partial rate constant for $MeAla^-$ does not further decrease does not necessarily contradict this possibility because this amino acid lacks the competitive C_α−H abstraction route. However, the small differences should not be overinterpreted.

The question as to what actual extent radicals **2**, **4** and **5** are formed via the various individual possible pathways, namely, direct abstraction (reactions 3, 5, and 6) or cage-mediated processes (reactions 7a,b, and 8) has to remain open at this point. Currently underway in this direction are studies on H/D isotope effects, which are expected to distinguish with less ambiguity between hydrogen abstraction and proton transfer. However, it is interesting to note that k(**4**) shows a little more variation than k(**6**) within the series Gly^-, Ala^-, and $MeAla^-$, where the ratios k(**4**):k(**6**) are 1.63, 1.86, and 2.43, respectively. This trend seems to imply an increase in the rate constant for the direct H abstraction (reaction 5) and/or an increase in the ratio of the rate constants for reactions 8 relative to 9 within the cage in that amino acid series. The latter, in turn, would reflect either a decrease in decarboxylation efficiency or an increase in proton-transfer efficiency.

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