Phenylalanine: Its OH and SO₄²⁻-Induced Oxidation and Decarboxylation. A Pulse Radiolysis and Product Analysis Study

Degui Wang, Heinz-Peter Schuchmann, and Clemens von Sonntag*
Max-Planck-Institut für Strahlenchemie, Stiftstraße 34, P. O. Box 101365, D-45413 Mülheim a. d. Ruhr
Z. Naturforsch. 48b, 761–770 (1993); received January 19, 1993
Amino Acid, Phenylalanine, Pulse Radiolysis, Peroxyl Radicals, Sulfate Radical

Phenylalanine has been oxidized by radiolytically generated hydroxyl and sulfate radicals. The ensuing intermediates and their reactions have been studied by pulse radiolysis and product analysis in the absence and presence of oxidants such as Fe(CN)₆³⁻ and O₂. Upon OH radical attack, hydroxycyclohexadienyl-type radicals are mainly formed while H-abstraction reactions can be neglected. In the presence of Fe(CN)₆³⁻ these radicals are for the most part oxidized to the corresponding tyrosines (80%), except for the ipso-OH-adduct radicals (= 20%). It is concluded that OH-addition is almost random, but with a slight avoidance of the meta-position relative to the ortho-, para- and ipso-positions. Oxygen adds reversibly to the OH-adduct radicals \(k_r = 1.8 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}\), \(k_e = 5.4 \times 10^4 \text{ s}^{-1}\). In this case, tyrosine formation occurs by HO₂-elimination. However, due to side reactions, tyrosine formation only reaches 52% of the OH radical yield. The tyrosine yield drops to 10% in the absence of an oxidant.

Upon SO₄²⁻-attack, decarboxylation becomes a major process (33% of SO₄²⁻) alongside the production of tyrosines (43%). Here, with Fe(CN)₆³⁻ as the oxidant the formation of p-Tyr (18.5%) and m-Tyr (16.5%) is preferred over o-Tyr formation (8.5%). It is believed that in analogy to other systems a radical cation is formed immediately upon SO₄²⁻-attack which either reacts with water under the formation of hydroxycyclohexadienyl-type ("OH-adduct") radicals, or decarboxylates after intramolecular electron transfer. The radical cation can also arise indirectly through H⁺-catalysed water elimination from the OH-adduct radicals. At pH 2 and a dose rate of 0.0046 Gy s⁻¹ CO₂ formation matches the OH radical yield when OH is the attacking radical. Below pH 2, \(G(CO_2)\) decreases with falling pH. This indicates the occurrence of another, unimolecular, pathway under these conditions competing effectively with decarboxylation. This appears to be a relatively slow deprotonation reaction of the carboxyl-protonated phenylalanine radical cation which gives rise to the benzyl-type radical.

Introduction

The OH-radical-induced hydroxylation of aromatic compounds has been extensively studied, especially by radiolysis techniques; for a review see [1], for aromatic amino acids see [2]. Due to its electrophilic nature the OH radical preferentially adds to the electron-rich aromatic ring. The hydroxycyclohexadienyl-type radicals thus formed are readily oxidized to phenols by strong oxidants such as Fe(CN)₆³⁻, with the obvious exception of the OH adducts at the position ipso to a given substituent [3–7].

The SO₄²⁻ radical is a very potent oxidant \((E_0 = 2.8 \text{ V}) [8]\) and has been proven to oxidize aromatic compounds [9–14] and DNA-nucleobases [15, 16] to the corresponding radical cations. Here as well, an adduct might be considered as a very short-lived intermediate. Indeed, in the reaction of SO₄²⁻ with certain olefins such SO₄²⁻ with certain olefins such SO₄²⁻-adducts are sufficiently long-lived to be detectable by e.s.r. spectroscopy [17, 18], though in the case of aromatic substrates the existence of such adducts is disputed (cf. [9, 12]).

The aromatic radical cations usually react with water, giving rise to hydroxycyclohexadienyl-type radicals. In the case of 1,3,5-trimethoxybenzene the radical cation predominates even at pH 7 [9, 19], but with less-electron-donating substituents acid is required to shift the equilibrium towards the radical cation [20, 21]. These radical cations are powerful oxidants and are able to extract an electron from the carboxylate group of a side chain, a reaction which leads to the decarboxylation of such an aromatic carboxylic acid [22].

The present work has been motivated by a report that the radiation-induced formation of o-tyrosine from phenylalanine [23] may be used as a marker to detect γ-irradiated food, especially meat [24–26]. Ionizing radiation causes the forma-
tion of radical cations from the substrate by the direct effect (energy absorbed by the protein under radical cation formation) and the formation of OH radicals from the solvent by the indirect effect (energy absorbed by the water surrounding the protein). As will be seen, both pathways may lead to phenylalanine-derived hydroxycyclohexadienyl radicals which then, in an additional oxidation step, are converted into the phenol. A second radical, transition metal ions, but also dissolved oxygen may function as the oxidant. The reaction of cyclohexadienyl-type radicals with oxygen is often complex, and oxygen addition to this kind of radical is often reversible (cf. [27]). In the case of phenylalanine, the relative yields of the various isomers (o-, m-, p-tyrosine) depend on whether these radicals are formed by OH radical addition or by radical cation hydration (for a brief report see [28]). The reaction of the radical cation with water competes effectively with its decarboxylation and the latter is quantitative only at a sufficiently low pH and low dose rate (long lifetimes of the radicals). When the carboxylate group becomes protonated the decarboxylation reaction is inhibited.

Substituted aromatic radicals are also capable by deprotonation to give rise to benzyl-type radicals [29]. This kind of reaction may compete with the decarboxylation pathway.

2. Experimental

Aqueous solutions (millimolar) of L-phenylalanine (Janssen) with various co-solutes (N₂O, t-butanol, K₂S₂O₈) to select the desired radical species for attacking the substrate (see next section) and to oxidize the substrate radicals (K₃Fe(CN)₆, O₂) were made up in Millipore Milli-Q purified water. Mixtures of gases (e.g. N₂O/O₂) at definite proportions except 80:20 v:v which is available commercially (Messer Griesheim) were prepared by mixing appropriate volumes of N₂O- and O₂-saturated solutions. The pH was adjusted using NaOH or HClO₄. γ-Rayolysis was carried out with a panoramic 60Co-γ-source at dose rates of 4.6 × 10⁻⁵ and 0.37 Gy s⁻¹. Electron pulse radiolysis was performed with a 2.8 MeV Van de Graaff electron generator delivering 0.4 μs electron pulses of 3–30 Gy. The UV spectra of the intermediates and their kinetics were observed, using a Xenon lamp that could be flashed for about 1 ms to achieve an approximately seventy-fold increase of the light intensity. Behind the pulse-radiolysis cell, the analysing light was focussed into a double monochromator followed by a photomultiplier.

The isomeric hydroxylated phenylalanines were determined by HPLC (column: 12.5 cm Nucleosil-RP-5-C18 5μm; eluent: water, 5 × 10⁻³ mol dm⁻³ KH₂PO₄, pH 5). CO₂ was determined by GC (column: Porapak N, 20 °C, carrier gas H₂, 0.9 bar inlet pressure) by flame ionization detection, after on-line catalytic reduction to methane [30].

3. Results and Discussion

Formation of radicals

Solvated electrons, OH radicals, and H-atoms are formed in the radiolysis of water (reaction (1)) [31, 32]. The solvated electrons may be converted with N₂O into further OH radicals (reaction (2)) or with peroxydisulfate into SO₄⁻ radicals (reaction (3)). In acid solution, additional H-atoms are formed at the expense of solvated electrons (reaction (4)). All these radicals react readily with phenylalanine (reaction (5), see below).

\[
\begin{align*}
\text{H}_2\text{O} & \xrightarrow{\text{ionizing radiation}} \text{e}_\text{aq}^- + \text{H}^+ + \text{H}_2\text{O}_2 \quad (1) \\
\text{e}_\text{aq}^- + \text{N}_2\text{O} & \rightarrow \text{OH} + \text{N}_2 + \text{OH}^- \quad (2) \\
\text{e}_\text{aq}^- + \text{S}_2\text{O}_8^{2-} & \rightarrow \text{SO}_4^{2-} + \text{SO}_3^- \quad (3) \\
\text{e}_\text{aq}^- + \text{H}^+ & \rightarrow \text{H}^- \quad (4)
\end{align*}
\]

\‘OH, SO₄⁻, H⁻, e⁻ + Phe → Phe-derived radicals

The phenylalanine-derived radicals may be reacted with additives such as Fe(CN)₆³⁻ or O₂. The use of a 4:1 mixture of N₂O/O₂ allows to convert essentially all of the solvated electrons into OH radicals while at the same time supplying a high enough concentration of O₂ to react rapidly with the Phe-ÖH-adduct radicals. Oxygen will also react with the H-atoms (k = 2 × 10⁻¹⁵ dm³ mol⁻¹ s⁻¹ [33]), yielding HO₂⁻.

The radiation chemical yields (G values) for the primary radicals [31, 32] are G(e⁻) = 2.75 × 10⁻⁷ mol J⁻¹, G(OH) = 2.8 × 10⁻⁷ mol J⁻¹ and G(H) = 0.57 × 10⁻⁷ mol J⁻¹. The so-called molecular products H₂O₂ (G(H₂O₂) = 0.73 × 10⁻⁷ mol J⁻¹) and H₂ (G(H₂) = 0.47 × 10⁻⁷ mol J⁻¹) do not interfere with the free radicals studied in this systems.

Hydroxyl-radical-induced reactions

In N₂O-saturated solutions above pH 3, the yield of the H-atoms is only about 10% of the OH-radical yield, and we therefore here neglect the reactions of the H-atom. By OH addition, four dif-
Different radicals can be formed (2–5, reactions (6)–(9)).

The possibility of an abstraction of the weakly bound benzylic hydrogen (formation of 6, reaction (10)) must be considered while H-abstraction at the amino acid carbon can be neglected: the rate constant of the OH radical with alanine is merely $7.7 \times 10^7 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ while it reacts with the phenylalanine zwitterion 1 with a rate constant of $k = 6.4 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}$ [33]. The transient UV/VIS absorption spectrum may provide information regarding the relative importance of these processes. Figure 1 shows the spectrum (cf. [34]) obtained from a N$_2$O-saturated aqueous solution containing $10^{-3} \text{ mol dm}^{-3}$ phenylalanine subjected to 2.7 MeV electron pulses of 0.4 $\mu$s duration and a dose of about 6 Gy. It is characterized by a strong absorption with $\lambda_{\text{max}} = 310–320 \text{ nm}$.

Benzyl radicals have sharp maxima at 257, 307 and 318 nm ($\varepsilon = 14000, 3300$ and $5500 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$, respectively) [29]. The OH-adduct radical (as well as the H-adduct radical) in the benzene system is characterized by a UV spectrum with a maximum at 310 nm of $\varepsilon(\text{OH-adduct}) = 3600 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [27] ($\varepsilon(\text{H-adduct}) = 4400 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ [35]). These data can be taken as a guide for assigning the phenylalanine-derived transient spectrum. On the basis of the 320 nm absorption alone, an estimate concerning a contribution of the benzyl radical cannot be made. But crucially, at about 260 nm there is no indication of any material contribution ($\leq 5\%$) of a species absorbing as strongly and with such a sharp maximum, as does the benzyl radical. The spectrum shown in Fig. 1 is practically indistinguishable from the one obtained with benzene under the same conditions [27], and thus we conclude that abstraction of benzylic H-atoms by the OH-radical (reaction (10)) is a minor process [34].

The OH-adduct radicals 3–5 yield the corresponding tyrosines upon oxidation. Since the ipso adduct 2 does not give rise to a tyrosine, the combined yield of the isomeric tyrosines represents a lower limit to the amount of OH-radical addition to the ring. Fe(CN)$_3^-$ is a very suitable oxidant to quantitatively convert the hydroxycyclohexadienyl radicals to the tyrosines [4, 36], and the ratios of the tyrosines in Table I column 3 therefore reflect the ratios of the OH-adduct radicals. As can be
seen from Table I 2nd column, under these conditions \( G(\text{total tyrosines}) \) accounts for about 80% of \( G(OH) \). The rest goes into \textit{ipso}-addition and perhaps a small amount of benzyl radical formation. The ratios of the three hydroxycyclohexadienyl radicals are very similar to those obtained in the case of toluene [7]. In an earlier pulse-radiolytic study of phenylalanine [34], a computational analysis of the kinetics of the change of the transient UV spectrum, somewhat different values for these ratios were arrived at (\( o:m:p = 1:0.3:0.6 \)), but owing to the difficulties inherent in that approach [34] we consider those results not to be in contradiction with ours.

In the hydroxylation of aromatic compounds, a 1,2-migration of deuterium and other substituents, away from the position of hydroxyl entry, has been observed (the so-called NIH shift [36–39]). This process apparently involves hydroxycyclohexadienyl carbocations. The migration of the hydroxyl group itself is also possible but plays a minor part [37]. Irrespective of mechanistic details, we may summarily write the tyrosine formation according to reactions (12), (13) and (14) and postulate that the yield of a given tyrosine reflects the distribution of the preceding precursor radicals. From this reasoning and the data in Table I (2nd column) it follows that OH-radical addition is only slightly selective, with a small preference for addition in the \textit{ortho}-positions (37%, 18.5% per site) and the \textit{para}-position (19%), while OH-radical addition in the \textit{meta}-positions appears a bit lower (24%, 12% per site). Addition to the \textit{ipso}-position (plus H-abstraction from the benzyl position) accounts for the remainder of 20%; any well-defined products from this pathway have as yet not been identified.

In the absence of an oxidant the tyrosine yields are much lower (Table I 1st column). This is readily understood since in a disproportionation reaction only one out of two precursor radicals is converted into a tyrosine molecule. Dimerization reactions also take place, as we know from the reactions of the unsubstituted cyclohexadienyl and hydroxycyclohexadienyl radicals [35, 40]. At low pH, \( \text{CO}_2 \) formation occurs (see below).

In \( \text{N}_2\text{O} / \text{O}_2 \)-saturated solutions, the OH-adduct radicals react rapidly with oxygen, but they do not disappear completely. An absorption remains, its size depending in an inverse manner on the oxygen concentration. This parallels the behaviour of the unsubstituted hydroxycyclohexadienyl radical (from OH plus benzene) where it could be shown that hydroxycyclohexadienyl radicals and oxygen are in equilibrium with the corresponding peroxyl radicals [27]. As expected for such a situation, a plot of \( k_{\text{obsd}} \) vs. \([\text{O}_2]\) gives a straight line (Fig. 2). From the slope, a forward-reaction rate constant \( k = 1.8 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) (this quantity is a composite since four different adduct radicals are being formed at the same time), and from the intercept whose size is essentially determined by the rate of the reverse reaction, an average reverse-reaction rate constant \( k = 5 \times 10^4 \text{ s}^{-1} \) is estimated. Their ratio yields a "composite" equilibrium constant of \( K = 3400 \text{ dm}^3 \text{ mol}^{-1} \) which is very close to that determined for the unsubstituted benzene system [27].
The analogy to the benzene system also holds for the subsequent reactions: the hydroxycyclohexadienyl radicals eliminate HO$_2^-$ and so give rise to the tyrosines (e.g. reactions (22), (24), and (26)). The kinetics of the HO$_2^-$-elimination can be followed by pulse radiolysis. The HO$_2^-$-radical is in rapid equilibrium with O$_2^-$(pK$_a$(HO$_2^-$) = 4.8), and tetranitromethane is used to monitor O$_2^-$ formation (reaction (28) which is fast with $k = 2 \times 10^9$ dm$^3$ mol$^{-1}$ s$^{-1}$) [41–43].

$$O_2^- + C(NO_2)_4 \rightarrow C(NO_2)_3^- + NO_2^- + O_2 \quad (28)$$

The buildup of the nitroform anion is first-order and is shown in Fig. 3 (inset).

The observed rate constant, this one again an overall quantity, is about $1.6 \times 10^3$ s$^{-1}$. Justification for treating the kinetic data (Figures 2 and 3) in this indiscriminate manner is drawn from the fact that the yields of the different tyrosines are similar (Table I, column 3).
Regarding the quantitative importance, under pulse-radiolysis conditions, of the \( \text{O}_2^{\cdot-} \)-forming reactions (22), (24), and (26), it can be calculated from the absorption coefficient of the nitroform anion (\( \epsilon(350 \text{ nm}) = 15000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} \)) that the yield of \( \text{HO}_2^{\cdot-} \) eliminated from the peroxyl radicals (\( G(\text{HO}_2^{\cdot-}) = 2 \times 10^{-7} \text{ mol J}^{-1} \)) extrapolated to zero dose; see Figure 3) is considerably lower than one would estimate if they all were to eliminate \( \text{HO}_2^{\cdot-} \). This low value is reflected in a correspondingly low tyrosine total yield (see Table I, column 3). In fact \( G(\text{tyrosines}) \) as determined after \( \gamma \)-radiolysis are about equal to \( G(\text{HO}_2^{\cdot-}) \), extrapolated to zero dose. At non-zero doses, because of the relatively slow \( \text{HO}_2^{\cdot-} \)-elimination, the bimolecular decay of the radicals \( (R' + \text{RO}_2 \rightarrow \text{RO}_2 + R'O_2) \) can no longer be neglected [45, 46]. Endoperoxide formation and subsequent ring fragmentation occur in the benzene system [45] in competition with \( \text{HO}_2^{\cdot-} \)-elimination [46]. Similar reactions must be expected in the present case, but here the fragment products have eluded identification as their structures are complex and the yields per individual product very low.

**SO\(_4^{\cdot-}\)**-induced reactions

The \( \text{SO}_4^{\cdot-} \) radical is well-known to oxidize aromatic compounds to the radical cation stage [9–14], which upon hydrolysis and further oxidation of the hydroxycyclohexadienyl radical (e.g. by higher-valent transition metal ions) gives rise to the corresponding phenol. Phenyl-substituted carboxylates suffer decarboxylation *via* intramolecular electron transfer to the radical cation [18]. \( \text{SO}_4^{\cdot-} \) attacks the carboxyl group directly much more slowly (by about two orders of magnitude) than the aromatic ring [18, 47]. For the present case, the rate constant of the reaction of \( \text{SO}_4^{\cdot-} \) with phenylalanine (reaction (5); cf. Scheme 3) has been measured at a pH of 4.4 by following the first-order decay of the \( \text{SO}_4^{\cdot-} \) absorption at 450 nm as a function of the phenylalanine concentration \( (3 \times 10^{-5} \sim 1 \times 10^{-4} \text{ mol dm}^{-3}) \). \( \epsilon(450) \) has been determined at 1360 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) which is bracketed by the values of 1100 and 1600 dm\(^3\) mol\(^{-1}\) cm\(^{-1}\) previously reported [5, 48].

From the linear \( k_{\text{obsd}} \) vs. \([\text{Phe}]\) plot (not shown) a bimolecular rate constant \( k(\text{SO}_4^{\cdot-} + \text{Phe}) = 2.7 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) is calculated. Thus it is ensured that at millimolar substrate concentrations, the formation of the initial organic transient will be essentially complete within a microsecond, and so does not interfere with the kinetics of the subsequent transformations. The solutions contained \( t \)-butanol at a concentration of 0.3 mol dm\(^{-3}\) to divert the OH radicals from reacting with the phenylalanine \( k(\text{OH} + t\text{-BuOH}) = 6 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \) [33]; \( k(\text{SO}_4^{\cdot-} + t\text{-BuOH}) = 4.0 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} \)

![Scheme 3](image)
Fig. 4. Evolution of the absorption spectrum produced in a solution containing phenylalanine (1 millimolar), K$_2$S$_2$O$_8$ (4 millimolar), and $t$-butanol (0.3 molar) (pH 4.4) by SO$_4$$. (•) 2 μs, (O) 60 μs, (▲) 1.4 ms after the pulse. Pulse dose, 3 Gy.

[49]. The spectral evolution of the intermediate(s) at pH 4.4 is shown in Fig. 4.

The absorption maximum immediately after the pulse is at 310 nm (A in Fig. 4) but shifts to 320 nm ($k = 2.2 \times 10^4$ s$^{-1}$). Apart from the shoulder at 350 nm (assignment see below), the main component of the resulting spectrum B is similar to that of the OH-adduct radicals whose absorption also peaks at 320 nm (Fig. 1). Therefore we assign B (disregarding the shoulder at 350 nm) to the combination of these OH-adducts. An estimate of the yield, based on the OH-adducts’ absorption coefficient (see above) and the absorbance at the maximum measured after 60 μs, comes out lower than expected if these precursor radicals, generated at the $G$ value of SO$_4$$^-$ (2.8 $\times$ 10$^{-7}$ mol J$^{-1}$; under the present conditions the H-atoms do not contribute to SO$_4$$^-$ production but are scavenged by the phenylalanine, cf. [33]), were to react exclusively with water to form these OH-adduct radicals even when the termination reactions of the latter have been taken into account.

Now looking at the $\gamma$-radiolysis, tyrosines are formed at only 43% of the SO$_4$$^-$ yield (Table I, column 4): in the presence of Fe(CN)$_6$$^{3-}$ which oxidizes some intermediate which could be the SO$_4$$^-$-adducts (unlikely, cf. [9]), or the hydrolysis product of the radical cation, the OH-adducts (likely), $G$(tyrosines) = $1.2 \times 10^{-7}$ mol J$^{-1}$. CO$_2$ is a further product, $G$(CO$_2$) = $0.9 \times 10^{-7}$ mol J$^{-1}$ or one third of the SO$_4$$^-$ yield. The production of CO$_2$ indicates that the radical cation is the crucial intermediate [18]. The ratios of the isomeric tyrosines obtained after SO$_4$$^-$ attack upon oxidation with Fe(CN)$_6$$^{3-}$ (Table I, 4th column) differ from those registered when these products are generated not from the hydrolysis products of the radical cation but from the directly-formed OH-adducts (Table I, 2nd column).

The pulse radiolysis experiments do not reveal whether the observed build-up ($k = 2.2 \times 10^4$ s$^{-1}$ at 320 nm) is due to the transformation of an SO$_4$$^-$-adduct into the radical cation (reaction (29), Scheme 3), followed by a much faster and hence kinetically invisible hydrolysis of the latter, or whether the spectral change directly reflects the hydrolysis of the radical cation. The moderately-fast rise-time of 30 μs would not in principle rule out the latter possibility, as there are examples of radical cations in aqueous solution with lifetimes such as this and longer: radical cations of certain thymine derivatives show lifetimes of 2–20 μs [15], those of methoxylated benzenes are stable against hydrolysis on the pulse radiolysis time-scale [9]. The photoionization of phenylalanine has recently been studied [50]. Spectroscopic data that would allow to discriminate between an SO$_4$$^-$-adduct and the radical cation have apparently never been reported. Moreover, the formation of radical cations from aromatics by the action of SO$_4$$^-$ has been considered as a very fast process, cf. [9, 12]. This implies that A in Fig. 4 probably reflects the radical cation.

The material balance, $G$(tyrosines) + $G$(CO$_2$) vs. $G$(SO$_4$$^-$), falls short by $0.7 \times 10^{-7}$ mol J$^{-1}$, i.e. 3–5 and 7 account for three quarters of the amount of SO$_4$$^-$ originally generated, leaving a share of about one quarter for the radicals 2 and 6, similar to the situation when the OH-radical is used as the attacking species. If it is assumed that none other than the radical cation is the intermediate, it follows that its hydrolysis is twice as fast as the intramolecular electron transfer (reaction (30)) from the radical-cation moiety to the carboxyl group, which gives rise to decarboxylation (reaction (31)). The latter is expected to be fast (cf. [18]) and it is therefore likely, given that a similar radical derived from trimethylamine shows a weak absorption at 350 nm [51], that some presence of the
α-aminoalkyl radical 8 causes the shoulder at 350 nm in the spectrum shown in Fig. 4, as it evolves with time.

**pH-Dependent decomposition of the OH-adduct radicals**

The radical cation can in principle also result from the H⁺-induced water elimination of the OH-adduct radical (reaction (−32)) [18, 52]. Hence one could expect an increasing CO₂ yield with increasing H⁺-concentration. As shown in Fig. 5 this is indeed the case.

However, G(CO₂) after having reached a maximum falls away again at still lower pH values. This behaviour can be rationalized if it is assumed that electron transfer (reaction (30)) followed by decarboxylation will only occur with the carboxyl function unprotonated. As the pH is lowered through the pKₐ of the radical cation, it becomes increasingly protonated at this function (the pKₐ of phenylalanine is at 1.83, and the analogous pKₐ of the radical cation is expected to lie not far below this value). A second unimolecular irreversible process, such as proton loss of the protonated radical cation 9a giving rise to the carboxyl-protonated benzyl radical 6a, could become predominant (cf. [18]) and explain the subsidence of G(CO₂) below pH 2. Radical-termination reactions cannot explain this fall-off since OH-adduct protonation (reaction (−37)) and proton-induced radical cation formation (reaction (39)) both become faster in equal proportion as the pH decreases (cf. Scheme 4).

![Scheme 4](image)

Fig. 5. G values of CO₂ produced in the γ-radiolysis of argon-saturated aqueous solutions of phenylalanine (1 millimolar) at different dose rates [(*) 4.6 × 10⁻³ Gy s⁻¹, (△) 0.37 Gy s⁻¹], as a function of pH.

Computational modeling of the situation described by Scheme 4 indicates that if the rate constant of decarboxylation (k₃₀−₃₁) assumes values in the order of 10⁴ s⁻¹, and k₄₁ values in the order of 10² s⁻¹ (radical-termination rate constants being assigned values in the order of 10⁹ dm³ mol⁻¹ s⁻¹), the findings shown in Fig. 5 can be reproduced in a qualitative manner. The fall-off of G(CO₂) below pH ~2 could thus be due to the slow formation of the benzyl radical, and its general reduction with the higher dose rate across the entire pH range, to the termination reactions. If the unimolecular...
reactions (30–31) and (41) were much faster than indicated, then the dose-rate dependence (which originates in the competition between reactions that are unimolecular and others that are bimolecular in radicals) would disappear since in the model the radical termination reactions have been chosen near the diffusion-controlled limit already and so leave no room for a further increase of their values. Moreover, an upper limit near $10^4 \text{s}^{-1}$ is imposed on the rate constant of the decarboxylation reaction by the observation (see above) of a time constant of $2.2 \times 10^4 \text{s}^{-1}$ for the decay of the radical cation (intermediate A, Fig. 4). It was not attempted to obtain a close fit as the number of the different radical species in this system is considerable and the values for the individual rate constants of self-termination and cross-termination reactions unknown.

The slowness of the reaction to form the benzyl radical would explain why it has not been observed in the pulse radiolysis experiments. Moreover, this reaction is apparently only shown by the carboxyl-protonated radical cation $9a$ which is denied decarboxylation. The latter route is open to the carboxyl-unprotonated radical cation $9$ which plays its part in the medium to weakly-acidic pH range. Once $9$ is formed, it decarboxylates rather effectively. This can be inferred from the close to quantitative CO$_2$ yield at pH 2 at low dose rates (relative to the maximum possible, cf. Fig. 5) upon γ-radiolysis, and is confirmed by the absence of any typical benzyl radical absorption after SO$_4^{-}$ attack in pulse radiolysis. Obviously, deporotonation of the radical cation $9$ at the benzyl function, a reaction that has been shown to be quite effective in the case of the toluene radical cation [29] is slow compared to decarboxylation and hydrolysis.

We thank the Volkswagen Foundation for financial support within the framework of its China programme (project I/64–964). D. Wang is grateful for a research stipend.