Reactions of 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulfonate) Dianion, $ABTS^{2-}$, with $^{\bullet}OH$, $(SCN)_2^{\bullet-}$, and Glycine or Valine Peroxyl Radicals

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$ABTS^{2-}$, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) dianion, was used as a reference to compare the reactivity of peroxyl radicals of two amino acids, glycine and valine, in aqueous solutions at natural pH. Peroxyl radicals were produced by pulse radiolysis and the product of their reaction with $ABTS^{2-}$ the $ABTS^{\bullet-}$ radical was observed spectrophotometrically. Experimental kinetic traces were fitted using chemical simulation. The rate constants of reactions of glycine and valine peroxyl radicals with $ABTS^{2-}$ were $(6.0 \pm 0.2) \times 10^6$ and $(1.3 \pm 0.1) \times 10^5$ $M^{-1}\cdot s^{-1}$, respectively. Moreover, it was found that only 60% of glycine radicals formed upon its reaction with $^{\bullet}OH$ radicals reacted with molecular oxygen to yield peroxyl radicals. Comparison of experimental data with simulations of chemical reactions in irradiated ABTS and ABTS/NaSCN solutions showed that $ABTS^{\bullet-}$ forms in the reaction with $^{\bullet}OH$ with a yield of 43% and rate constant of $(5.4 \pm 0.2) \times 10^9$ $M^{-1}\cdot s^{-1}$ and in the reaction with $(SCN)_2^{\bullet-}$ with a yield of 57% and rate constant of $(8.0 \pm 0.2) \times 10^8$ $M^{-1}\cdot s^{-1}$.

Introduction

Recent evidence suggests that proteins are the main initial biological targets for attack by $^{\bullet}OH$.[1−5] This results in the formation of radical sites on the protein backbone and amino acid residues. These, in turn, may readily accept molecular oxygen to form peroxyl radicals able to propagate peroxidation processes and thus should be regarded as a form of reactive oxygen species.[2] Knowledge of the reactivity of amino acid peroxyl radicals, and other products of amino acid oxidation, is essential for the understanding of the mechanisms of many processes proceeding in living organisms exposed to oxidative stress or conditions such as aging.[6,7] Comparison of the reactivities of peroxyl radicals can be made with molecules having low reduction potentials and giving colored oxidation products easily quantified spectrophotometrically. One such reference molecule is $ABTS^{2-}$, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) dianion, which has a reduction potential of 0.63 V.[8] This compound is usually used in the form of ammonium salt and thus is present in water solution as a dianion. Upon one electron oxidation a radical is formed, denoted as $ABTS^{\bullet-}$. This species exhibits an absorption spectrum in the visible range peaking at 415, 650, and 735 nm, with the relatively high absorbance coefficients of 36 000, 13 500, and 15 000 M^{-1} cm^{-1}, respectively.[8] The usefulness of $ABTS^{\bullet-}$ as a reference radical has been shown by Wolfenden and Willson already in 1982,[9] and it has been used extensively since.[10−14]

So far, no detailed mechanisms of the reaction of oxidizing radicals with $ABTS^{2-}$ have been published. In this study, we carried out pulse radiolytic measurements of the kinetics of reactions of $^{\bullet}OH$ and $(SCN)_2^{\bullet-}$ with $ABTS^{2-}$. The results were used to compare the relative reactivities of glycine and valine peroxyl radicals utilizing $ABTS^{2-}$ as a reference compound. These two amino acids were chosen because they were reported to give very high (valine) and very low (glycine) yields of hydroperoxides upon γ irradiation.[1] Peroxyl radicals are the direct precursors of hydroperoxides and it seemed likely that differences in the reactivities of the amino acid peroxyl radicals can be responsible for the amounts of stable hydroperoxides they generate.

TABLE 1: Absorption Coefficients of the Species Included in the Kinetic Simulation

absorbing species	λ, nm	ϵ, $M^{-1}cm^{-1}$	reference
$ABTS^{\bullet-}$	650	13 500	8
$ABTS^{\bullet-}$	475	2200	8
$ABTS^{\bullet-}$	415	35 000−36 000	8, 23
$(SCN)_2^{\bullet-}$	475	7600	24
$(SCN)_2^{\bullet-}$	650	610	24

Experimental Section

Glycine (Sigma), valine (Sigma), ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) diammonium salt (Sigma), and NaSCN (Fluka) were used as received. Water from MilliQ Plus (Millipore) was used throughout. All experiments were carried out at natural pH (5.5−6) and a temperature of 23 ± 1 °C.

The pulse radiolysis system at the Institute of Applied Radiation Chemistry based on the linear accelerator delivering 6 MeV electrons has been described elsewhere.[15] Electron pulses of 3, 7, or 17 ns delivered doses of ca. 5, 18, or 50 Gy, respectively, as measured with a KSCN dosimeter.[16] Samples were saturated with appropriate gases directly in the Suprasil spectrophotometric cell prior to the irradiation.

The solutions were saturated with N_2O (25 mM) to convert hydrated electrons into $^{\bullet}OH$ radicals or with the mixture N_2O/O_2 (4:1 v/v) to allow formation of peroxyl radicals. In some cases, saturation with O_2 (1.4 mM) was used to check whether the simulations performed fitted the kinetic traces recorded in the system when a mixture of reducing and oxidizing radicals formed in the irradiation. It is important to note that in all of the systems presented in this paper the hydrated electron decayed within 200 ns and hence did not contribute to the absorption at 650 nm in the microsecond time scale.

Kinetic simulations were done with the use of Chemical Kinetics Simulator 1.01 (IBM Almaden Research Center). The $^{\bullet}OH$ radical and hydrated electron yields were taken as 0.28

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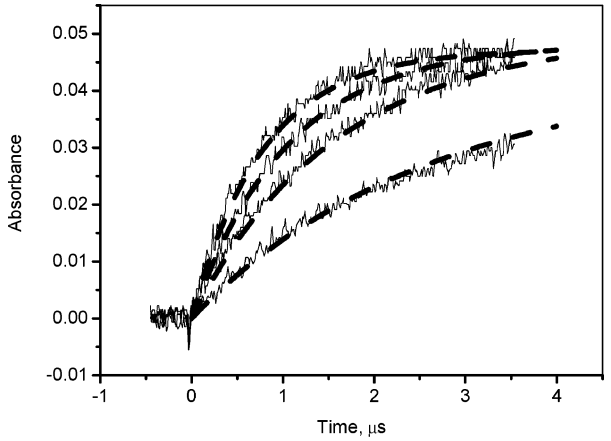

Figure 1. Transient absorption traces at 415 nm in pulsed, N_2O-saturated aqueous $ABTS^{2-}$ solutions at natural pH. Concentrations of $ABTS^{2-}$ were 0.03, 0.05, 0.075, and 0.1 mM from the bottom trace to the top one. Pulse length, 3 ns; dose, ~5 Gy. Dashed lines represent simulated kinetic curves (see the text).

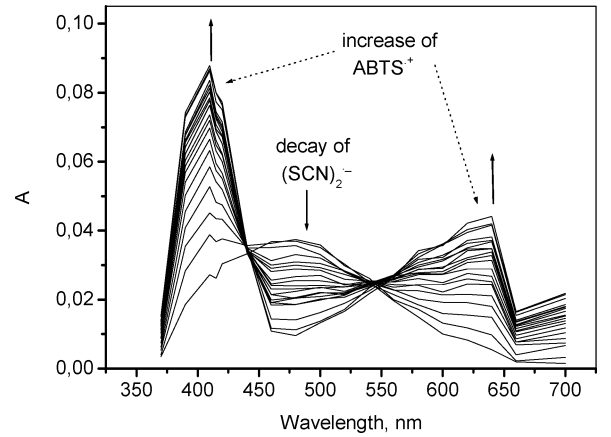

Figure 2. Transient absorption spectra in pulsed, N_2O-saturated aqueous solutions containing 50 mM NaSCN and 1 mM ABTS shown in the time span from 0 to 20 μs after the pulse. Up arrows show the increase of the absorption of peaks of $ABTS^{\bullet-}$ at 415 and 650 nm. The down arrow shows the decay of the absorption of $(SCN)_2^{\bullet-}$ at 475 nm. Pulse length, 7 ns; dose, ~18.5 Gy.

and 0.34 μmol/J, respectively.[16,17] To compare simulations with the experimental data, the appropriate absorbance coefficients were used, as given in Table 1. Absolute error limits for pulse radiolysis experiments are about ±10%. Our experiments were repeated 3−5 times, and the error limits given in the abstract refer to the scatter of results. The simulations were sensitive to the changes of rate constants at a much lower level of around 1%.

Results and Discussion

Reactions with $^{\bullet}OH$ Radical. The transient absorption spectrum observed in pulse-irradiated, N_2O-saturated aqueous solution of ABTS shows three maxima peaking at ~415, ~650, and ~735 nm characteristic for the $ABTS^{\bullet-}$ radical. Under these conditions, the radical forms upon one electron oxidation of the $ABTS^{2-}$ anion with $^{\bullet}OH$ radicals. However, the amount of the $ABTS^{\bullet-}$ radical equaled only half of the amount of $^{\bullet}OH$ radicals produced upon radiolysis. The rest probably attacked other sites of the $ABTS^{2-}$ molecule than the thiazoline nitrogen atom, forming transients transparent in the visible range. Such parallel reactions of the hydroxyl radical with different sites of the same molecule were observed not only for $ABTS^{2-}$ but also, for example, with phenothiazines.[9,18]

Measurements of the rise of the $ABTS^{\bullet-}$ absorption at 415 and 650 nm for different concentrations of ABTS, chosen to ensure pseudo-first-order kinetics, allowed us to evaluate the overall rate constant of reaction of $^{\bullet}OH$ radicals with $ABTS^{2-}$ as 1.27×10^{10} $M^{-1}\cdot s^{-1}$. This compares well with the 1.2×10^{10} $M^{-1}\cdot s^{-1}$ reported by Wolfenden and Willson.[9] Figure 1 shows the experimental traces taken at 650 nm and fitted using kinetic simulation. The simplified reaction scheme consistent with this result is made up of the following reactions, where k_1 is taken from Buxton et al.[19] and the other rate constants are our estimates:

$$e_{aq}^{-} + N_2O \rightarrow {}^{\bullet}OH + OH^{-} + N_2 \qquad k_1 = 9.1 \times 10^9\ M^{-1}\cdot s^{-1} \quad (1)$$

$$^{\bullet}OH + ABTS^{2-} \rightarrow OH^{-} + ABTS^{\bullet-} \qquad k_2 = 5.4 \times 10^9\ M^{-1}\cdot s^{-1} \quad (2)$$

$$^{\bullet}OH + ABTS^{2-} \rightarrow (^{\bullet}OH\text{-}ABTS^{2-}) \qquad k_3 = 7.3 \times 10^9\ M^{-1}\cdot s^{-1} \quad (3)$$

$$(^{\bullet}OH\text{-}ABTS^{2-}) + ABTS^{\bullet-} \rightarrow \text{prod} \qquad k_4 = 1.0 \times 10^7\ M^{-1}\cdot s^{-1} \quad (4)$$

$$^{\bullet}OH + ABTS^{\bullet-} \rightarrow OH^{-} + ABTS \qquad k_5 = 1.0 \times 10^9\ M^{-1}\cdot s^{-1} \quad (5)$$

Note that the sum of the rate constants $k_2 + k_3$ gives the overall rate constant for the reaction of $^{\bullet}OH$ with $ABTS^{2-}$ (1.27×10^{10} $M^{-1}\cdot s^{-1}$) according to the scheme of two parallel pseudo-first-order reactions ($ABTS^{2-}$ in excess over $^{\bullet}OH$), from which one obtains

$$[ABTS^{\bullet-}] = A_0(1 - \exp(-(k_2 + k_3)[ABTS^{2-}]t)) \quad (6)$$

where $A_0 = k_2[^{\bullet}OH]_0/(k_2 + k_3)$. Reactions 4 and 5 were included to account for the consumption of $ABTS^{\bullet-}$, although in the time range shown in Figure 1 their role is negligible. However, reaction 4 may be responsible for the significantly shorter lifetime of the radiolytically produced ABTS radical as compared to that produced chemically. The second step of the oxidation of $ABTS^{2-}$ is also known from purely chemical experiments with $S_2O_8^{-}$ (used in excess) as an oxidizing agent.[20] We estimated the probable high rate constant of reaction 5, which, together with k_4, was used successfully in further simulations (see the following paragraphs).

Reactions with $(SCN)_2^{\bullet-}$ Anion Radical. To check the validity of the above simulation, we applied it to aqueous solutions containing $ABTS^{2-}$ and SCN^{-}. The relative concentrations of these solutes were adjusted to ensure at least 50-fold excess of the SCN^{-}. Pulse irradiation of these solutions under N_2O saturation results in quantitative conversion of the $^{\bullet}OH$ radicals into $(SCN)_2^{\bullet-}$ anion radicals, which in turn oxidize $ABTS^{2-}$ to the $ABTS^{\bullet-}$. The recorded absorption spectra confirmed these expectations as can be seen in Figure 2. However, the yield of the $ABTS^{\bullet-}$ radical was only about 60% of the $(SCN)_2^{\bullet-}$ anion radicals generated. This can be readily estimated taking the highest absorption value at 475 nm (for the $(SCN)_2^{\bullet-}$) and the lowest at 650 nm (for the $ABTS^{\bullet-}$) and using the respective molar absorptivities. To account for this, reaction 10 was included in the kinetic simulations. At present, we have no information on the nature of the product of this

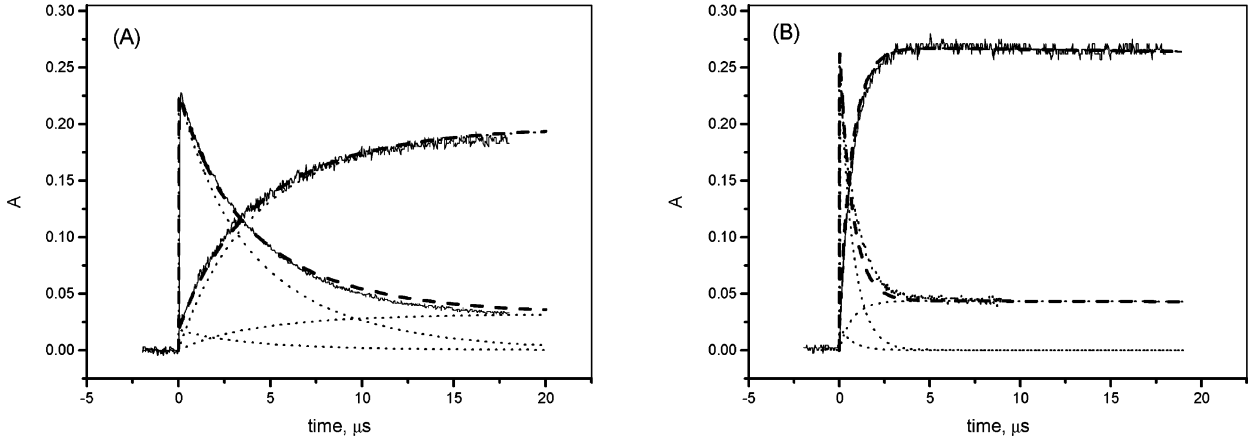

Figure 3. Transient absorption traces at 475 nm (the decay of $(SCN)_2^{\bullet-}$) and 650 nm (the increase of $ABTS^{\bullet-}$) in pulsed, N_2O-saturated aqueous ABTS solutions at natural pH. (A) [ABTS] = 0.15 mM. (B) [ABTS] = 1 mM. Pulse length, 17 ns; dose, 50−60 Gy. Dotted lines represent the contribution of the absorption of both observed species at 475 and 650 nm. Dashed lines are the sum of these contributions and represent the simulated kinetic curves (see the text).

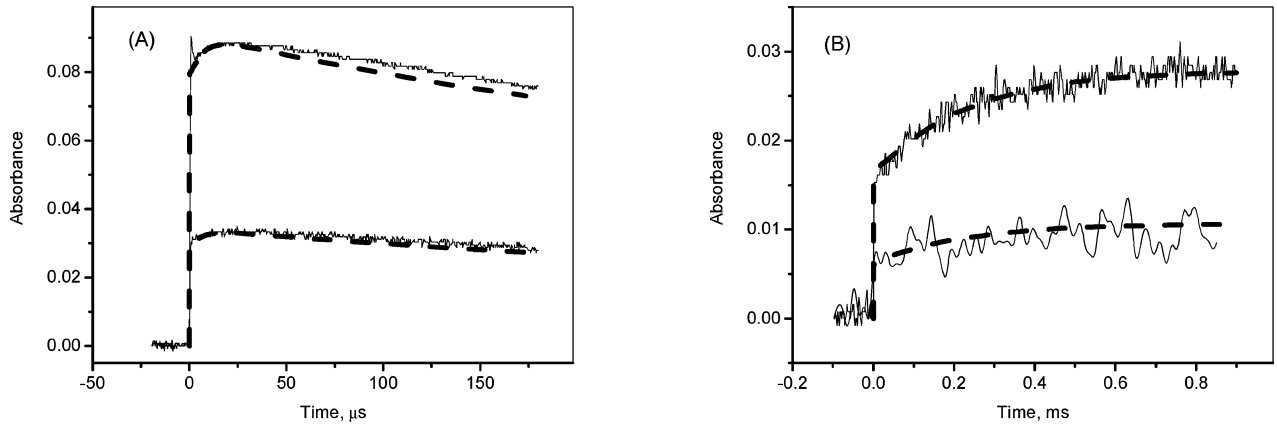

Figure 4. Transient absorption traces at 415 nm (upper curves) and 650 nm lower curves) in pulsed, N_2O/O_2-saturated aqueous solutions at natural pH containing 1 mM ABTS and 100 mM glycine (A) or valine (B). Pulse length, 7 ns; dose, ~18.5 Gy. Dashed lines represent simulated kinetic curves (see the text).

reaction. The full scheme included reactions 7−11 in addition to 1−5 listed in the previous paragraph.

$$^{\bullet}OH + SCN^- \rightarrow OH^- + SCN^{\bullet} \qquad k_7 = 1.4 \times 10^{10}\ M^{-1}\cdot s^{-1} \quad (7)$$

$$SCN^{\bullet} + SCN^- \rightarrow (SCN)_2^{\bullet-} \qquad k_8 = 9.9 \times 10^9\ M^{-1}\cdot s^{-1} \quad (8)$$

$$(SCN)_2^{\bullet-} + ABTS^{2-} \rightarrow 2SCN^- + ABTS^{\bullet-} \qquad k_8 = 8.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (9)$$

$$(SCN)_2^{\bullet-} + ABTS^{2-} \rightarrow \text{prod} \qquad k_9 = 6.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (10)$$

$$2\,(SCN)_2^{\bullet-} \rightarrow (SCN)_2 + 2SCN^- \qquad k_{10} = 1.2 \times 10^9\ M^{-1}\cdot s^{-1} \quad (11)$$

The rate constants k_7 and k_8 were taken from the recent work of Milosavljevic and LaVerne,[21] and k_{11} was taken from Elliot and Sopchyshyn.[22]

Figure 3 shows the experimental traces taken at 475 and 650 nm together with curves fitted by kinetic simulation. The sum of the rate constants of reactions 9 and 10 gives the value $1.4 \times 10^9\ M^{-1}\cdot s^{-1}$, which is the overall rate constant of the reaction of thiocyanate dimer radical anions with $ABTS^{2-}$ obtained by us and agrees with the value of $1.5 \times 10^9\ M^{-1}\cdot s^{-1}$ given by Wolfenden and Willson.[9] As can be seen, our simulations correspond closely with the experimentally observed traces, both for the decay of thiocyanate dimer radical anions (475 nm) and for the formation of $ABTS^{\bullet-}$ radicals (650 nm) in the range of ABTS concentrations from 0.15 up to 1 mM. To obtain this fit, absorptions of $ABTS^{\bullet-}$ at 475 nm and of $(SCN)_2^{\bullet-}$ at 650 nm had to be taken into account (Table 1).

Reactions with Glycine and Valine. Two aliphatic aminoacids, glycine and valine, were chosen for these studies because they show the lowest (3%) and the highest (49%) yield of hydroperoxides formed upon reaction with hydroxyl radicals in aqueous solution.[1] Because the amino acid peroxyl radicals are precursors of the hydroperoxides, our aim was to test whether their reactivitites toward $ABTS^{2-}$ may be different. As demonstrated by the results shown in Figure 4, this was indeed the case for pulse-irradiated solutions saturated with N_2O/O_2 and containing excess of glycine or valine over the $ABTS^{2-}$. In the systems saturated with N_2, no increase of the absorption at 415 nm or 650 nm was observed. This observation means that neither valine nor glycine radicals are able to oxidate $ABTS^{2-}$ to its radical; therefore, these processes were not included to the reaction schemes. Instead, the formation of complexes between amino acid radicals and $ABTS^{\bullet-}$ was taken into account by the analogy to the complexes formed by alkyl

radicals.[25,26] The latter add to the ring position of $ABTS^{\bullet-}$, and the process proceeds with the rate constant on the order of 10^9 $M^{-1}\cdot s^{-1}$.[26]

The much lower initial yield of the $ABTS^{\bullet-}$ radicals in the presence of valine can be readily explained by the significant difference of the rate constants of the reactions of $^{\bullet}OH$ radicals with these amino acids; the rate constant is nearly 5 times higher for valine (7.6×10^8 $M^{-1}\cdot s^{-1}$)[19] than for glycine (1.7×10^7 $M^{-1}\cdot s^{-1}$),[19] and hence more $ABTS^{\bullet-}$ radicals were produced directly upon oxidation with $^{\bullet}OH$ radicals in the presence of glycine.

To simulate the kinetic trace for valine, the following processes were included in addition to reactions 1−5 (k_{12} is taken from Buxton et al.,[19] and all other rate constants are our current estimates):

$$e_{aq} + O_2 \rightarrow O_2^{\bullet-} \qquad k_{12} = 1.9 \times 10^9\ M^{-1}\cdot s^{-1} \quad (12)$$

$$^{\bullet}OH + Val \rightarrow OH^- + Val^{\bullet} \qquad k_{13} = 1.2 \times 10^9\ M^{-1}\cdot s^{-1} \quad (13)$$

$$Val^{\bullet} + O_2 \rightarrow ValOO^{\bullet} \qquad k_{14} = 2.0 \times 10^9\ M^{-1}\cdot s^{-1} \quad (14)$$

$$ValOO^{\bullet} \rightarrow O_2^{\bullet-} + prod \qquad k_{15} = 4.0 \times 10^3\ s^{-1} \quad (15)$$

$$ValOO^{\bullet} + ABTS^{2-} \rightarrow ValOO^- + ABTS^{\bullet-} \qquad k_{16} = 1.3 \times 10^5\ M^{-1}\cdot s^{-1} \quad (16)$$

$$Val^{\bullet} + ABTS^{\bullet-} \rightarrow Complex \qquad k_{17} = 5.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (17)$$

The rate constant of the reaction of the $^{\bullet}OH$ radical with valine obtained from our simulation, reaction 14, is higher than those (8.5×10^8 $M^{-1}\cdot s^{-1}$ at pH 6.9) quoted by Prütz and Vogel[27] or that (6.6×10^8 $M^{-1}\cdot s^{-1}$ at pH 6.6) given by Masuda et al.,[28] but it is very close to the value (1.4×10^9 $M^{-1}\cdot s^{-1}$) measured in our laboratory in competitive pulse radiolysis determinations with ascorbic acid.[29] One should note that the old results were obtained using steady-state X or γ radiolysis. The rate constants for the formation of valine peroxyl radicals (reaction 14) as well as for the formation of glycine peroxyl radicals (reaction 22) are assumed to be the same as those for the reaction of alkyl radicals with molecular oxygen.[30] The rate constant for the unimolecular decay of the valine peroxyl radical with release of $O_2^{\bullet-}$ (reaction 15) is our current estimate. The reported rate constant for the unimolecular decay of the glycine peroxyl radical is 1.5×10^5 s^{-1} (reaction 23).[31] Judging from the low yield of glycine hydroperoxides, one should expect glycine peroxyl radicals to be less stable than those of valine. However, because the use of the above set of reactions did not give a sufficiently close fit of theory and experiment, we included the possibility of $ABTS^{\bullet-}$ formation from the reaction of hydrated electrons with $ABTS^{2-}$:

$$e_{aq}^- + ABTS^{2-} \rightarrow X^{\bullet} + prod \qquad k_{18} = 1.5 \times 10^9\ M^{-1}\cdot s^{-1} \quad (18)$$

$$X^{\bullet} + ABTS^{2-} \rightarrow X^- + ABTS^{\bullet-} \qquad k_{19} = 2.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (19)$$

These reactions have been proposed by Wolfenden and Willson[9] to explain the observation that the characteristic absorption band of $ABTS^{\bullet-}$ radicals were seen in N_2-saturated solutions containing *t*-butanol. We checked that the contribution of reactions 18 and 19 was negligible in the simulations concerning either ABTS or ABTS/KSCN solutions in water. The simulation including reactions 1−5 and 12−19 gave a very good fit to the experimental data, as shown in Figure 4B.

We applied the same model for the N_2O/O_2-saturated glycine solutions, substituting only the rate constants of glycine with $^{\bullet}OH$ radicals and the rate constants for the other reactions of glycine radicals. It appeared that all rational combinations of rate constants led to an overestimation of the absorption observed at either 415 or 650 nm. Thus, we took into account the possibility that the attack of the hydroxyl radical on the glycine molecule can lead to three types of radicals: α-amino-α-carboxyl, α-aminoalkyl (after decarboxylation), and aminyl radical.[32] The first two are carbon-centered radicals, and the latter is nitrogen-centered. According to Štefanić et al.,[33] these three radicals form in the ratio 1:0.6:1 at high pH when glycine is in an anionic form. Because there are strong suggestions that N-centered radicals have short lifetimes[32] and hence do not lead to $ABTS^{2-}$ oxidation, we included in the reaction scheme two ways in which $^{\bullet}OH$ radicals may react with glycine; the first would give C-centered radicals, denoted here as $GlyC^{\bullet}$, able to form peroxides (reaction 20), and the second way included all other types of radicals, denoted here as $GlyN^{\bullet}$ (including N-centered radicals), not able to form peroxides (reaction 21). The reaction set used for simulation of glycine solutions is then as follows (reactions common for valine and glycine systems are not shown here), where k_{23} is taken from Abramovitch and Rabani[31] and all other rate constants are our current estimates:

$$^{\bullet}OH + Gly \rightarrow OH^- + GlyC^{\bullet} \qquad k_{20} = 1.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (20)$$

$$^{\bullet}OH + Gly \rightarrow OH^- + GlyN^{\bullet} \qquad k_{21} = 6.5 \times 10^7\ M^{-1}\cdot s^{-1} \quad (21)$$

$$GlyC^{\bullet} + O_2 \rightarrow GlyOO^{\bullet} \qquad k_{22} = 2.0 \times 10^9\ M^{-1}\cdot s^{-1} \quad (22)$$

$$GlyOO^{\bullet} \rightarrow O_2^{\bullet-} + prod \qquad k_{23} = 1.5 \times 10^5\ s^{-1} \quad (23)$$

$$GlyOO^{\bullet} + ABTS^{2-} \rightarrow GlyOO^- + ABTS^{\bullet-} \qquad k_{24} = 6.0 \times 10^6\ M^{-1}\cdot s^{-1} \quad (24)$$

$$GlyC^{\bullet} + ABTS^{\bullet-} \rightarrow prod \qquad k_{25} = 1.0 \times 10^7\ M^{-1}\cdot s^{-1} \quad (25)$$

$$GlyN^{\bullet} + ABTS^{\bullet-} \rightarrow prod \qquad k_{26} = 8.0 \times 10^8\ M^{-1}\cdot s^{-1} \quad (26)$$

Figure 4A shows that simulation based on this mechanism gave a good fit to the experimental data. Štefanić et al.[33] found that direct attack at the nitrogen atom is the dominant reaction of the hydroxyl radical with glycine anions. Our findings strongly suggest that for glycine zwitterions about 60% of $^{\bullet}OH$ radicals attack at carbon atoms to form $GlyC^{\bullet}$-type radicals, in agreement with the results obtained with direct rapid-flow EPR method.[34]

To ascertain the validity of the proposed mechanism, we applied it to results obtained with oxygen-saturated aqueous solutions of valine or glycine. The results shown in Figure 5A and B support the mechanism.

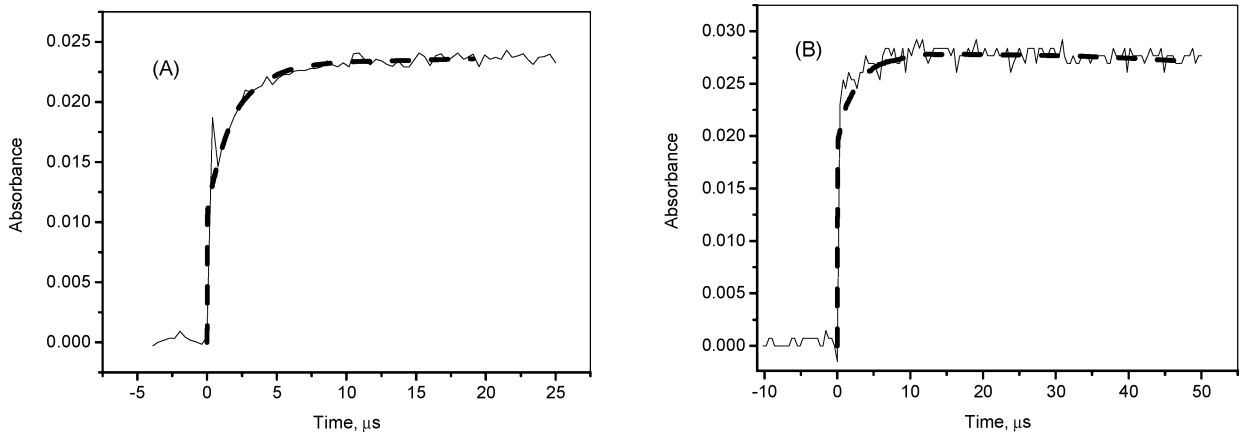

Figure 5. Transient absorption traces at 650 nm in pulsed, O_2-saturated aqueous solutions at natural pH containing 1 mM ABTS and 100 mM glycine (A) or valine (B). Pulse length, 7 ns; dose, ~18.5 Gy. Dotted lines represent simulated kinetic curves (see the text).

Note, however, that although the rate constant of the reaction of the •OH radical with glycine derived from our simulation ($k_{20} + k_{21} = 1.65 \times 10^8$ $M^{-1}\cdot s^{-1}$) is significantly higher than that (8.9×10^6 $M^{-1}\cdot s^{-1}$ at pH 4.7) published by Štefanić et al.[33] or that (1.7×10^7 $M^{-1}\cdot s^{-1}$ at pH 5.9) given by Scholes et al.,[35] it agrees much better with that (2.5×10^8 $M^{-1}\cdot s^{-1}$) obtained in our laboratory in competitive pulse radiolysis determinations with the use of ascorbic acid[29] or with that (8.33×10^8 $M^{-1}\cdot s^{-1}$ at pH 1) published by Pramanick et al.[36]

Conclusions

$ABTS^{2-}$ in water solutions reacts with •OH radicals simultaneously by one electron oxidation, giving the strongly absorbing radical $ABTS^{\bullet-}$, and by •OH addition and/or •H abstraction, giving products with no absorption in the visible region. The rate constants determined in this study are 5.4×10^9 and 7.3×10^9 $M^{-1}\cdot s^{-1}$, respectively. The sum of these rate constants gives the value 1.27×10^9 $M^{-1}\cdot s^{-1}$, which equals the overall rate constant of the reaction of $ABTS^{2-}$ with •OH, as determined by us experimentally and in agreement with published value.[9]

The new rate constants were used to simulate the kinetics of fast reactions observed in the aqueous system containing excess SCN^- anions over $ABTS^{2-}$. The simulation showed that thiocyanate dimer radical anions also oxidize $ABTS^{2-}$ in at least two ways: one leads to the $ABTS^{\bullet-}$ via one electron oxidation, and the other leads to products transparent in the visible range. The good fit between the simulated curves and experimental traces allowed us to determine the rate constants of the electron transfer and addition/abstraction reactions of the $(SCN)_2^{\bullet-}$ to be 8×10^8 and 6×10^8 $M^{-1}\cdot s^{-1}$, respectively. Again, the sum of these two values gives the overall rate constant of the reaction of $ABTS^{2-}$ with $(SCN)_2^{\bullet-}$ radical anions of 1.4×10^9 $M^{-1}\cdot s^{-1}$, as measured here and in agreement with published values.[21]

Comparison of the simulated and experimental curves gave the values 1.3×10^5 $M^{-1}\cdot s^{-1}$ and 6.0×10^6 $M^{-1}\cdot s^{-1}$ for the rate constants of reaction of the peroxyl radicals of valine and glycine with $ABTS^{2-}$, respectively. Furthermore, we concluded that the unimolecular decay of the valine peroxyl radical into the superoxide radical anion and oxidation products of valine had a rate constant of 4.0×10^3 s^{-1}, which is significantly lower than the rate constant for the analogous decay of the glycine peroxyl radical (1.5×10^5 s^{-1}).[31] Our investigations have lead us to the other interesting finding that the reaction of hydroxyl radicals with glycine at natural pH (5.5−6) gives ca. 60% radicals able to react rapidly with molecular oxygen to form peroxyl radicals. These radicals are probably C-centered ones, while the rest are N-centered.

The dramatic difference in the hydroperoxide yields reported for glycine and valine can now be explained by the lower yield of glycine peroxyl radicals and their lower stability, with faster unimolecular decay. One may therefore regard glycine as having relatively low ability to propagate the peroxidation chain, which is a very important feature because glycine is very common amino acid.

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