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Pulse radiolysis studies of intermolecular charge transfers involving tryptophan and three-electron-bonded intermediates derived from methionine

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Abstract The oxidation processes of the radiation-generated, three-electron-bonded intermediates AcMet$_2$ $[\mathrm{S}\therefore\mathrm{S}]^+$ and AcMet $[\mathrm{S}\therefore\mathrm{Br}]$ were investigated by pulse radiolysis via their reactions with tryptophan (TrpH). These intermediates were derived from *N*-acetyl-methionine amide (*N*-AcMetNH$_2$) and *N*-acetyl-methionine methyl ester (*N*-AcMetOMe). The bimolecular rate constant *k* of the reaction between each intermediate and L-tryptophan (TrpH) was measured. For *N*-AcMetNH$_2$, *k* for the reaction of AcMet$_2$ $[\mathrm{S}\therefore\mathrm{S}]^+$ with TrpH were 3.4×10^8 and 2.2×10^8 dm^3 mol^{-1} s^{-1} at pH = 1 and 4.5, respectively. For *N*-AcMetOMe, *k* for the reaction of AcMet$_2$ $[\mathrm{S}\therefore\mathrm{S}]^+$ with TrpH were 4.0×10^8 and 2.8×10^8 dm^3 mol^{-1} s^{-1} at pH 1 and 4.5, respectively. The rate constants for the intermolecular transformation of Met $[\mathrm{S}\therefore\mathrm{Br}]$ into TrpH$^+$ or Trp were also estimated. For *N*-AcMetNH$_2$, *k* for the reaction of AcMet$_2$ $[\mathrm{S}\therefore\mathrm{Br}]$ with TrpH were 2.6×10^8 and 3.3×10^8 dm^3 mol^{-1} s^{-1} at pH 1 and 4.5, respectively. Related mechanisms were discussed.

Keywords Three-electron-bonded radicals · Methionine derivatives · Tryptophan · Intermolecular electron transfer · Pulse radiolysis

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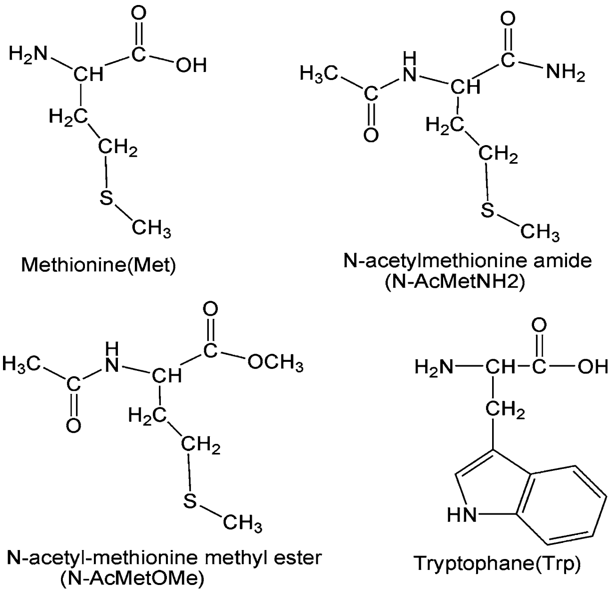
Introduction

The possibility of forming sulfur–sulfur and sulfur–halogen three-electron bonds may be particularly relevant to oxidation processes in biological systems. Sulfur-containing radical cations and odd-electron species are markedly unreactive toward molecular oxygen [1–3]. Recently, a peptide containing a methionine (Met) residue in the C-terminal domain has been deemed important for its neurotoxicity and its propensity to reduce transition metals [4]. For small Met-containing peptides, the one-electron oxidation of an N- or C-terminal Met yields sulfide radical cations. These cations can stabilize via the respective formation of sulfur–nitrogen or sulfur–oxygen three-electron bonds [5–8]. However, the nucleophilic sulfur atom in the side chain of Met is very susceptible to oxidation. Therefore, Met is considered to play a key role in the migration of unpaired electrons in peptides and proteins [9]. Consequently, the damage caused by oxidizing radicals in such biological units may appear at positions different from the initial attack site. This phenomenon may result in a change in the native conformation of the protein and in the loss of enzymatic activity. In particular, the oxidation of Met in *β*-amyloid peptides, which are the major constituents of senile plaques in Alzheimer's disease, has been associated with the neurotoxicity of these sequences [10]. *N*-acetyl-L-methionine amide (*N*-AcMetNH$_2$) and *N*-acetyl-L-methionine methyl ester (*N*-AcMetOMe) (Scheme 1) are simple models of the amino acid Met incorporated in a peptide.

Pulse radiolysis studies by Hiller et al. [11] and Chu et al. [12] revealed that the oxidation of Met and (*N*-diisopropyloxyphosphoryl)methionine occurs readily. Oxidative attack always takes place at the sulfur atom and results in the formation of



Methionine(Met)

N-acetylmethionine amide (N-AcMetNH2)

N-acetyl-methionine methyl ester (N-AcMetOMe)

Tryptophane(Trp)

Scheme 1 Chemical structures of methionine derivatives three-electron-bonded species of the types Met_2 [S∴S]$^+$ or Met [S∴X] (X = Cl, Br, etc.). Bobrowski and Lubis [13] as well as Prutz et al. [14] investigated the intermolecular electron transfers involved in three-electron-bonded species derived from the oxidation of Met and dipeptides. The dipeptides included Met_2 [S∴S]$^+$, Met [S∴X]-Gly, as well as tryptophan (Trp), tyrosine (Tyr), and other amino acids. Other studies demonstrated that intramolecular electron transfers can occur within a (phosphoryl) dipeptide, or within polypeptides such as Met–Trp and Met–(X)n–Tyr (X = Pro, etc., n = 1–5) [12, 15, 16]. The rate of transformation of methionyl into tryptophyl radicals were also shown to depend on peptide concentration. This dependence indicated the occurrence of both inter- and intramolecular processes, which is in contrast with tryptophyltyrosyl peptides. These findings may lead to observable intermolecular charge transfer reactions within the products formed by oxidative attacks on free Met with Trp and Tyr. In the present work, pulse radiolysis was used to demonstrate the oxidizing nature of various intermediates of *N*-AcMetNH$_2$ and *N*-AcMetOMe via the oxidation of Trp in a free aqueous solution. The reaction was examined at different pH levels. Electron transfer processes involving Trp and the sulfur-containing three-electron-bonded intermediates were investigated.

Experimental

Materials

N-acetyl-L-methionine amide (*N*-AcMetNH$_2$), *N*-acetyl-L-methionine methyl ester (*N*-AcMetOMe), and L-tryptophan (TrpH) were obtained from Sigma and were used without further purification. All other compounds, which were of the highest commercially available purity, were from Wako Co., Japan. The pH values of amino acid solutions were adjusted by adding appropriate amounts of $HClO_4$, NaOH, or NaH_2PO_4.

Apparatus

The pulse radiolysis experiments were performed at the University of Tokyo using an electron linear accelerator (energy = 35 MeV; duration = 20 ns) coupled with an absorption spectroscopic detection system. Absorbed doses were determined by a KSCN dosimeter, which involved taking $G \times \varepsilon_{(SCN)2\bullet-} = 5.1 \times 10^4$ m^2/J [17] for N_2O-saturated 1×10^{-2} mol dm^{-3} potassium thiocyanate solutions. The analyzing light came from a 150 W xenon lamp (Hungarian Tungsram), which was pulsed for 3 ms to increase the intensity. Optical absorption was determined with a Bausch-Lomb monochromator, a Hamamatsu R 928 photomultiplier, and a Tektronix 7834 oscilloscope. The oscilloscope monitored the light passing through the irradiation quartz cell with an optical path of 20 mm. The signals were digitized using an Iwatsu 8123 digital oscilloscope and were stored in a private microcomputer for further analysis. The microcomputer software converted the data into give optical densities as well as first and second order kinetic parameters. Further details on the pulse radiolysis equipment are given elsewhere [18]. For measuring absorption spectra, a higher dose (ca. 35 Gy/pulse) was used. For determining rate constants, a lower dose (10–13 Gy/pulse) was applied.

Methods

All solutions were prepared with triple-distilled water at room temperature. Before irradiation, the solutions (pH 1) were saturated in high purity Ar for about 30 min to remove oxygen, and in high purity N_2O (pH $>$ 3) to convert e_{aq}^- into hydroxyl (OH) radicals. Hydrogen atoms, which cannot easily be converted into OH radicals at pH $<$ 3 (even in the presence of N_2O) reacted with Met at a rate constant k of $\sim 10^9$ dm^3 mol^{-1} s^{-1} [19]. However, their reaction products did not interfere with the optical signals observable in our system because earlier optical measurements showed only weak absorption below 300 nm ($\varepsilon_{280} = 200$ dm^3 mol^{-1} cm^{-1} [20] in acidic Met solutions).

Results and discussion

$\mathrm{Met_2}$ $[\mathrm{S}\therefore\mathrm{S}]^+$ as an oxidant

The transient absorption spectra formed by an $^\bullet$OH-induced oxidation in a model system containing 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ at pH 1 are shown in Fig. 1. The UV band at λ ca. 290 nm is generally characteristic of a-thioalkyl radicals (····-$^\bullet$CH-S-····). This band resulted from the deprotonation of the $R_2S^{\bullet+}$-type radical cation or from the direct H-atom abstraction by $^\bullet$OH radicals. The broad absorption spectrum with λ max = 480 nm is assigned to the three-electron-bonded dimer intermediate $\mathrm{Met_2}$ $[\mathrm{S}\therefore\mathrm{S}]^+$ [20, 21]. This band was formed via OH addition to the sulfur atom in the initial step (Reaction 1):

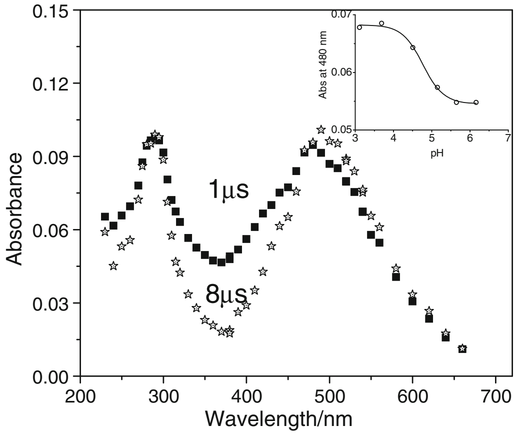


Fig. 1 Transient absorption spectra of an aqueous solution containing 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ saturated with N_2O at pH 1. Dose = 34 Gy/pulse. *Inset* The absorbance at 480 nm at different pH values

$$^{\bullet}\mathrm{OH} + \mathrm{Met} \rightarrow \mathrm{Met}\ [\mathrm{S}\therefore\mathrm{OH}] \tag{1}$$

Under the experimental conditions, the OH adducts underwent the elimination of $^{\bullet}$OH with the assistance of external protons (Reaction 2). A subsequent intermolecular equilibration occurred with a second unattacked Met molecule (Reaction 3). The transient absorption at 480 nm was varied by pH values, as shown in Fig. 1 (inset). $\mathrm{p}K_\mathrm{a}$ was determined to be 4.5 ± 0.2.

$$\mathrm{Met}\ [\mathrm{S}\therefore\mathrm{OH}] + \mathrm{H}^{+} \rightarrow \mathrm{Met}\ [\mathrm{S}^{+\bullet}] + \mathrm{H_2O} \tag{2}$$

$$\mathrm{Met}\ [\mathrm{S}^{+\bullet}] + \mathrm{Met} \leftrightharpoons \mathrm{Met_2}[\mathrm{S}\therefore\mathrm{S}]^{+} \tag{3}$$

The transient absorption spectra formed by an OH-induced oxidation in a model system containing 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ and 2×10^{-4} mol dm^{-3} Trp at pH 1 was shown in Fig. 2A. The concentration of AcMet was much higher than that of TrpH, and the rate constant of Met was k (OH + Met) = 2.3×10^{10} dm^3 mol^{-1} s^{-1} [11] and k (OH + TrpH) = 1.25×10^{10} dm^3 mol^{-1} s^{-1} [22]. Hence, more than 90% of the OH radicals were expected to react with Met. In our experiments, the dimer radical cation formation was completed within 1 μs pulse. About 15 μs after the electron pulse, a new transient species with characteristic absorptions at 325 and 570 nm [see spectrum (O)] was fully developed. This species was the tryptophyl radical cation (TrpH$^+$) [23], and its development was indicated by the identical growing and decaying processed observed at either 320 or 570 nm.

The characteristic absorption bands of the protonated tryptophyl radical cation at 325 and 570 nm were fully developed 15 μs after the pulse, even though the 325 nm

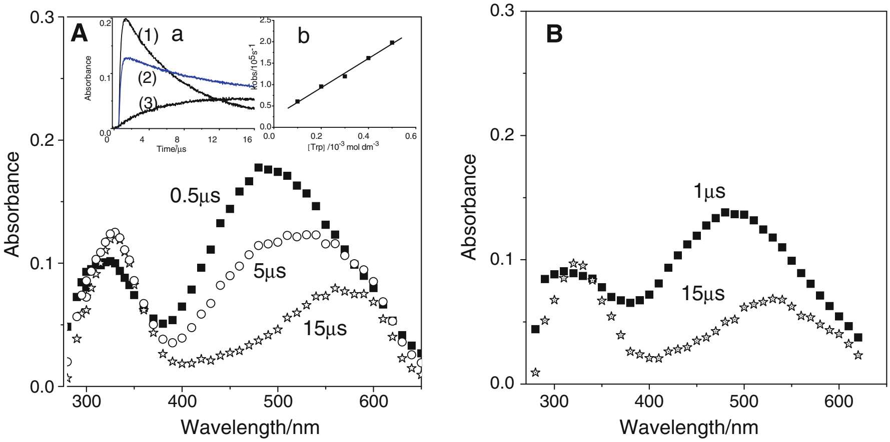


Fig. 2 Transient absorption spectra of 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ and 2×10^{-4} mol dm^{-3} Trp aqueous solutions saturated with Ar at **A** pH 1 and **B** pH 4.5. Dose = 34 Gy/pulse. *Inset a* The transient absorption curves observed at (1) 480, (2) 570 nm, and (3) obtained by subtracting (1) from (2) at 570 nm. *Inset b* The plot of observed k against TrpH concentration for the decay of the Met$_2$ [S∴S]$^+$ radical cation recorded at 480 nm

absorption was also discernible in the spectrum at 0.5 μs. The formation of $\mathrm{TrpH^{+\bullet}}$ at this time was only seen below 400 nm, where it absorbed more strongly than $\mathrm{Met_2\ [S\therefore S]^+}$. These results proved the occurrence of a fast OH-induced intermolecular charge transfer ($\mathrm{Met_2\ [S\therefore S]^+ \rightarrow TrpH}$). The markedly enhanced decay at 480 nm absorption in the presence of Trp also confirmed the oxidation of Trp by a sulfur-centered radical cation. The decay at 480 nm, which occurred exponentially in the absence of TrpH, was found to be faster with increased Trp concentration ($0.5\text{–}2 \times 10^{-4}$ mol dm^{-3}). A simplified mechanism of the $\mathrm{Met_2\ [S\therefore S]^+}$ decay is summarized as follows:

$$\mathrm{Met\ [S^{+\bullet}] \rightarrow Met\ [-H]^{\bullet} + H^+} \tag{4}$$

$$\mathrm{Met_2[S\therefore S]^+ +\ TrpH\ [COOH,\ NH_3^+] \rightarrow 2\ Met\ +\ TrpH^+} \tag{5}$$

By subtracting the contribution of $\mathrm{Met_2\ [S\therefore S]^+}$ from $\mathrm{TrpH^+}$ at 570 nm, the dimer intermediate $\mathrm{Met_2\ [S\therefore S]^+}$ was confirmed to decay at the same rate as the rate constant of the tryptophyl cation radical formation [Fig. 2A, inset a (3)]. The experimental rate constant was obtained by following the decay of $\mathrm{Met_2\ [S\therefore S]^+}$ at 480 nm. From Fig. 2A, inset b, a rate constant was determined to be 3.4×10^8 dm^3 mol^{-1} s^{-1}, which is basically consistent with Bobrowski's results [24]. Reaction (5) may only represent an overall reaction if the more reactive species is the molecular radical cation AcMet $\mathrm{[S^{+\bullet}]}$ in equilibrium with the dimer. The data presented in the present article did not allow us to distinguish between the two oxidizing species because all data have been recorded at the same Met derivative concentrations. The rate constant k_5 may be exclusively attributed to the reaction of three-electron-bonded species if the rate constants remained unaffected when the Met derivative concentrations were varied.

To compare the oxidation rate constants of both forms of Trp (e.g., zwitterionic TrpH $\mathrm{[COO^-, NH_3^+]}$ and fully protonated TrpH $\mathrm{[COOH, NH_3^+]}$), $\mathrm{Met_2\ [S\therefore S]^+}$ was designed to be generated at a pH higher than the pK_a of the Trp carboxyl group.

Upon an increased system pH of 4.5 ± 0.2, the characteristic absorption of $\mathrm{TrpH^{+\bullet}}$ at 580 nm shifted to a lower wavelength (530 nm; Fig. 2). This shift resulted from the contribution of the neutral radical $\mathrm{Trp^{\bullet}}$, which was transferred from TrpH $^{+\bullet}$ via deprotonation at p$K_a = 4.3$ [25].

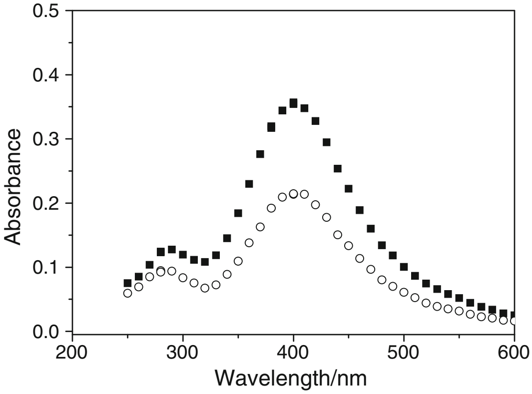
A similar kinetic picture of $\mathrm{AcMetNH_2}$ at pH 1 was obtained at pH 4.5. The bimolecular rate constant for Reaction (6) was determined by the kinetic analysis of the $\mathrm{Met_2\ [S\therefore S]^+}$ decay at 480 nm ($k_6 = 2.2 \times 10^8$ dm^3 mol^{-1} s^{-1}).

$$\begin{array}{r} \mathrm{Met_2\ [S\therefore S]^+ + TrpH\ [COO^-, NH_3^+] \rightarrow 2\ Met + TrpH^{+\bullet}} \\ \downarrow\quad \\ \mathrm{Trp^{\bullet} + H^+} \end{array} \tag{6}$$

$\mathrm{Met_2\ [S\therefore Br]}$ as an oxidant

An oxidized sulfur center can be stabilized not only as a $\mathrm{S\therefore S^+}$ radical cation complex, but also as a $\mathrm{S\therefore X}$ three-electron-bonded to a halogen atom (X = Cl or Br). After the pulse radiolysis of $\mathrm{N_2O}$-saturated aqueous solutions containing

Fig. 3 Transient absorption spectra of 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ and 5×10^{-2} mol dm^{-3} KBr in N$_2$O-saturated aqueous solutions at pH 4.3 recorded 1 (*filled square*) and 8 (*open circle*) µs after the pulse



2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$ and 0.05 mol dm^{-3} KBr at pH 4.3, a transient species with a maximum absorption at 400 nm was obtained (Fig. 3). This absorption was assigned to the three-electron-bonded intermediate AcMet [S∴Br]$^\bullet$, consistent with the results of Hiller et al. [26]. A simple reaction mechanism could be described as follows:

$$^\bullet\mathrm{OH} + \mathrm{Br}^- \rightarrow \mathrm{Br}^\bullet + \mathrm{OH}^- \tag{7}$$

$$\mathrm{Br}^\bullet + \mathrm{Br}^- \rightarrow \mathrm{Br}_2^{\bullet-} \tag{8}$$

Subsequently, the quantitative reaction of $\mathrm{Br}_2^{\bullet-}$ with Met derivatives could be described as:

$$\mathrm{Br}_2^{\bullet-} + \mathrm{Met} \rightarrow \mathrm{Met}\ [\mathrm{S}\therefore\mathrm{Br}]^\bullet + \mathrm{Br}^- \tag{9}$$

The rate constants of the formation of AcMet [S∴Br]$^\bullet$ were determined as $k_9 = 6.8 \times 10^8$ mol dm^{-3} s^{-1} at pH 2. k_9 was deduced from a series of the observed decay rates of the radical anion $\mathrm{Br}_2^{\bullet-}$ at different *N*-AcMetNH$_2$ concentrations. At pH 10, k was 6.4×10^8 mol dm^{-3} s^{-1} for *N*-AcMetNH$_2$ and was 7.5×10^8 mol dm^{-3} s^{-1} for *N*-AcMetOMe.

Subsequently, the oxidation of Trp by Met [S∴Br] was investigated in N$_2$O-saturated solutions containing 5×10^{-2} mol dm^{-3} KBr, 2×10^{-3} mol dm^{-3} AcMetNH$_2$, and up to 2×10^{-4} mol dm^{-3} Trp at pH 1.

Given that k ($\mathrm{Br}_2^{\bullet-}$ + Met) > k ($\mathrm{Br}_2^{\bullet-}$ + TrpH) [26] and the Trp to Met concentration ratio was ≤ 0.1, the direct oxidation of TrpH by $\mathrm{Br}_2^{\bullet-}$ was almost negligible. Therefore, any formation process observed after the electron pulse at longer wavelengths (e.g., 580 nm) and at wavelengths identical with the initial decay of Met [S∴Br] (e.g., 400 nm) can be attributed to the formation of the TrpH$^{+\bullet}$ radical cation. This formation was via the direct oxidation of the Trp molecule by Met [S∴Br] (Reaction 10):

$$\mathrm{Met}\ [\mathrm{S}\therefore\mathrm{Br}] + \mathrm{TrpH} \rightarrow \mathrm{Met} + \mathrm{Br}^- + \mathrm{TrpH}^{+\bullet} \tag{10}$$

The absorption spectra measured 2 and 20 µs after the pulse radiolysis of 2×10^{-3} mol dm^{-3} *N*-AcMetNH$_2$, 2×10^{-4} mol dm^{-3} Trp, and 5×10^{-2} mol dm^{-3} KBr in N$_2$O-saturated aqueous solutions at pH 1 was shown in Fig. 4.

Although there is some indication of a weak absorption band at around 320 nm, the spectrum at 2 μs is presumably caused by Met [S∴Br]. However, the spectra after 20 μs were identical to the spectra of the Met solutions in Fig. 2. In agreement with previous assignments, these spectra after 20 μs are attributed to Trp radical cations. Accordingly, Reaction (10) indeed proceeded with $k_{10} = 2.6 \times 10^8\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$.

Upon an increased pH of 4.50, the 580 nm absorption of the tryptophyl radical shifted to lower wavelengths (530 nm; Fig. 5). This shift was consistent with the known effect of pH on the transient absorption maxima of tryptophyl species. The rate of the intermolecular oxidation of the Trp zwitterionic form by Met [S∴Br] was practically the same with the fully protonated molecule ($k_{11} = 3.3 \times 10^8\ \mathrm{dm^3\ mol^{-1}\ s^{-1}}$).

$$\begin{array}{l} \text{Met [S}\therefore\text{Br] + TrpH [COO}^-\text{, NH}_3^+\text{]} \rightarrow \text{TrpH}^{+\bullet} + \text{Met} + \text{Br}^- \\ \qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad \downarrow \\ \qquad\qquad\qquad\qquad\qquad\qquad\quad \text{Trp}^\bullet + \text{H}^+ \end{array} \tag{11}$$

Finally, the rate constants of the intermolecular electron transfers from Trp to the three-electron-bonded intermediates are listed in Table 1. The intermediates were derived from the oxidation of $\mathrm{AcMetNH_2}$ and AcMetOMe by both $^\bullet$OH radicals and $\mathrm{Br_2^{\bullet-}}$ radical anions in aqueous solutions at different pH values. Apparently, intermolecular charge transfers within peptides containing Met and aromatic amino acids such as Trp occurred very rapidly. Similarly efficient intramolecular charge

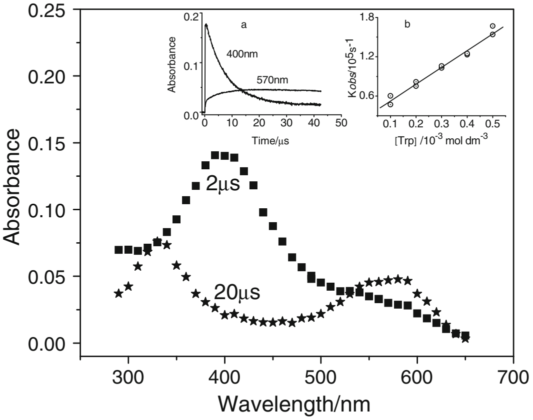


Fig. 4 Transient absorption spectra of $2 \times 10^{-3}\ \mathrm{mol\ dm^{-3}}$ *N*-$\mathrm{AcMetNH_2}$, $2 \times 10^{-4}\ \mathrm{mol\ dm^{-3}}$ Trp, and $5 \times 10^{-2}\ \mathrm{mol\ dm^{-3}}$ KBr in $\mathrm{N_2O}$-saturated aqueous solutions at pH 1 recorded 2 (*filled square*) and 20 (*star*) μs after the pulse at ~35 Gy/pulse. *Inset a* The transient absorption curves for the decay of $\mathrm{Met_2}$ [S∴Br] at 400 nm and the formation of $\mathrm{TrpH^+}$ at 570 nm. *Inset b* The plot of the observed decay rate constants of the transient species $\mathrm{Met_2}$ [S∴Br] (400 nm) against Trp concentrations at pH 1 (~12 Gy/pulse)

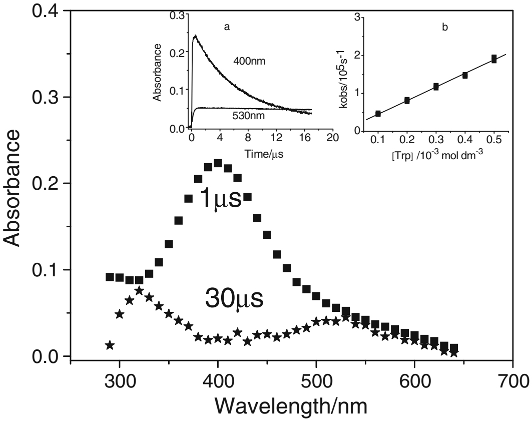


Fig. 5 Transient absorption spectra of 2 × 10^{-3} mol dm^{-3} *N*-AcMetNH$_2$, 2 × 10^{-4} mol dm^{-3} Trp, and 5 × 10^{-2} mol dm^{-3} KBr in N_2O-saturated aqueous solutions at pH 4.4 recorded 1 (*filled square*) and 30 (*star*) μs after the pulse. *Inset a* The transient absorption curves for the decay of Met$_2$ [S∴Br] at 400 nm and the formation of TrpH$^+$ at 530 nm. *Inset b* The plot of the observed decay rate constants of transient species Met$_2$ [S∴Br] (400 nm) against tryptophan concentrations at pH 4.4 (~12 Gy/pulse)

Table 1 The rate constants of the intermolecular electron transfers from Trp to AcMteNH$_2$ and to AcMetOMe in aqueous solutions

	k (mol dm^{-3} s^{-1})					
Initiator	AcMetNH$_2$		AcMetOMe		Met[a]	
pH	1	4.3	1	4.5	1	4.3
$^{\bullet}$OH	3.42 × 10^8	2.2 × 10^8	4.0 × 10^8	2.8 × 10^8	3.8 × 10^8	
$Br_2^{\bullet -}$	2.6 × 10^8	3.3 × 10^8	3.2 × 10^8	3.6 × 10^{8}[b]	4.6 × 10^8	4.6 × 10^8

[a] From Bobrowski [24]

[b] pH 5.5

transfers with rate constants in the order of 10^2–10^4 s^{-1} were also found to occur between Trp and Tyr residues in numerous synthetic and natural peptides [27, 28].

Conclusions

Intermolecular electron transfers from Trp to three-electron-bonded intermediates derived from the oxidation of *N*-AcMetNH$_2$ and *N*-AcMetOMe by $^{\bullet}$OH and $Br_2^{\bullet -}$ radicals were investigated. The reactions took place in N_2O-saturated aqueous solutions with pH values of 1.0 – 4.5. A series of rate constants for the intermolecular electron transfers were determined. The results revealed that Trp–Met pairs can form very favorable redox couples in biological systems, which is consistent with earlier reports of more efficient and rapid intramolecular charge transfers in synthetic as well as natural peptides containing Met.

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