'OH radical Induced Decarboxylation of Methionine-containing Peptides. Influence of Peptide Sequence and Net Charge

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The 'OH radical induced oxidation of methionine-containing peptides results in significantly different decarboxylation yields upon variation of the location of the methionine unit with respect to the terminal functions (Met–Gly, Met–Glu, Met–Gly–Gly, Gly–Met–Gly, Gly–Met and Gly–Gly–Met), or with the nature of neighbouring amino acids located at the *N*-terminus of methionine (Ala–Met, β-Ala-Met, Val-Met, Leu–Met, Ser–Met, Thr–Met, His–Met, γ-Glu–Met, Pro–Met, Gly–Gly–Phe–Met and Tyr–Gly–Gly–Phe–Met). The CO_2 yields measured in γ-radiolysis vary from 0% (Met–Gly, Met–Glu, Met–Gly–Gly, Gly–Met–Gly, and Pro–Met) to about 80% (γ-Glu–Met) of the 'OH radicals available. Mechanistically, the decarboxylation is considered to proceed *via* an intramolecular 'outer sphere' electron transfer from the methionine carboxylate function to the oxidized sulphur function $>S^{\bullet+}$. An additional *N*-terminal decarboxylation route exists in γ-Glu–Met which requires assistance by the α-positioned free amino group. Both processes compete with deprotonation of $>S^{\bullet+}$ at the carbon atom α-positioned to sulphur. The relative rates of all these competing pathways, and consequently the decarboxylation yields, are shown to depend on (i) the electron inductive properties of substituent groups at the α-carbon of the *N*-terminal amino acid, (ii) the net electric charge of the peptide molecule, and (iii) the distance between the centres of positive charge ($-NH_3^+$ and $>S^{\bullet+}$).

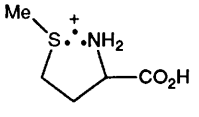
Methionine residues, due to the unique combination of hydrophobicity and nucleophilic reactivity provided by the thioether group in the side chain, participate in the maintenance of the structure of protein molecules, form coordination bonds with metal ions, take part in transfer of electrons, and are also part of substrate binding sites.[1] The nucleophilic sulphur atom in the side chain of methionine is very susceptible to oxidation. Therefore, methionine was considered to play a key role in the migration of unpaired electrons in peptides and proteins.[2,3] Consequently, the damage caused by oxidizing radicals in such biological units may appear at positions different from the initial site of attack. This may result in a change of the native conformation of the protein, and in the loss of enzymatic activity.[1]

Previous pulse radiolysis studies have shown that hydroxyl radicals are most effective oxidants for sulphide functions.[4–7] These investigations have also revealed that the reaction of 'OH with organic sulphides does not proceed *via* a straightforward, 'outer sphere' electron transfer but primarily yields an OH-adduct, $>{}^{\bullet}S{-}OH$, which may then convert into the molecular $>S^{\bullet+}$ or a complex $(>S\therefore S<)^+$ radical cation. Both cationic species are known to exist in the general equilibrium shown in eqn. (1). Electronically the complex cation is characterized by a

$$R_2S^{\bullet+} + R_2S \rightleftharpoons (R_2S\therefore SR_2)^+ \qquad (1)$$

2-centre-3-electron ($2\sigma/1\sigma^*$) bond between the interacting heteroatoms.

Stabilization of $>S^{\bullet+}$ may also be achieved by association of the unpaired p electron with a free p electron pair of a heteroatom, X, other than sulphur, *e.g.* nitrogen, oxygen, halogen *etc.* to yield $>S\therefore X$ type species.[6–9] An S∴N bonded, short lived intermediate has been identified in the oxidation of methionine and suggested to play a key role in the 'OH radical induced decarboxylation of this amino acid.[5,7] An important feature in the stabilization of the S∴N bond is the possibility of establishing a five-membered ring configuration as shown in structure **1**.



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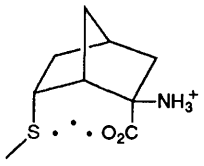
The appearance of CO_2 as a stable product with a parallel decrease in the yield of the intermolecular S∴S bonded radical cation is the most characteristic feature in the 'OH radical induced oxidation of methionine in going from acid (pH 0) to almost neutral (pH 5–6) solutions[5,7,10] suggesting that the decarboxylation and the S∴S bond formation are competitive. A similar conclusion evolves from recent work[11] on the 'OH radical induced oxidation of *exo*-2-(amino)-*endo*-6-(methylthio)bicyclo[2.2.1]heptane-*endo*-2-carboxylic acid in which the sulphur and carboxylate function are kept in rigid *endo* positions relative to each other. In this case it appears that CO_2 formation competes with sulphur–carboxylate bond formation, the latter being depicted in structure **2**. The relative ratio between decarboxylation and formation of **2** has been found to depend on pH.

From previous investigations it has emerged that decarboxylation of amino acids can generally be initiated either directly by oxidation of the carboxylate or indirectly *via* oxidation of the amino group.[5,7,9,11–14] The actual route seems often to be



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controlled by geometric parameters, particularly when the oxidation occurs through an intramolecular process involving an oxidizing radical centre remote from the location of the amino and carboxy functions. This is true, for example, in the case of the 'OH induced oxidation of methionine,[5,7] its norbornane derivative, *exo*-2-(amino)-*endo*-6-(methylthio)bicyclo[2.2.1]heptane-*endo*-2-carboxylic acid[11] or *S*-methyl cysteine[12] where the primarily oxidized sulphur arranges with either the amino nitrogen or the carboxy oxygen in transient 5- and 6-membered ring structures.

It has been inferred[12] that the presence of activating groups (*e.g.* amino or hydroxy) strongly enhances the decarboxylation process owing to their ability to delocalize the unpaired electron in the resulting (α-amino or α-hydroxyl) radical. Thus, decarboxylation was not observed upon the 'OH radical induced oxidations of organic acids lacking amino groups or with amino groups not located α to the carboxylate.[11-13]

Derivatization of either the amino or carboxy group in amino acids may also hamper the decarboxylation process quite effectively as indicated, for example, by a significantly reduced CO_2 yield from *N*-acetyl methionine as compared to methionine.[5] This result is corroborated by a corresponding higher yield and greater stability of the S.∴S centred dimeric complex.[5] Particularly long-lived transients of this kind have also been observed upon pulse radiolysis of slightly acidic to neutral solutions of methionine ethyl ester[15] and L-methionylglycine.[15,16]

The present study has been undertaken to elucidate the prerequisites for decarboxylation of methionine-containing peptides by varying their composition and the relative location of the individual amino acids (including two enkephalins[17]). Structural parameters are expected to particularly affect those processes which possibly compete with decarboxylation. This refers, for example, to the association of an oxidized sulphur of the methionine moiety into an S.∴S-bonded dimeric radical cation or intramolecular sulphur–carboxylate interaction species (see structure **2**). Similarly instructive should be the location of charged functionalities in the peptides since they may influence the rates of deprotonation of $>S^{\bullet+}$ type intermediates which probably serve an important function in the overall decarboxylation mechanism. Finally, it has been anticipated that this investigation would provide further support for a generalization of the conclusions recently drawn from a single example amino acid (parent compound of **2**),[11] particularly in view of the question by which mechanism decarboxylation is initiated.

Experimental

The peptides of L-isomeric configuration: glycyl–methionine (Gly–Met), glycyl–glycyl–methionine (Gly–Gly–Met), methionyl–glycine (Met–Gly), methionyl–glutamic acid (Met–Glu), methionyl–glycyl–glycine (Met–Gly–Gly), γ-glutamyl–methionine (γ-Glu–Met), β-alanyl–methionine (β-Ala–Met), glycyl–glycyl–phenylalanyl–methionine (Gly–Gly–Phe–Met) and tyrosyl–glycyl–glycyl–phenylalanyl–methionine (Tyr–Gly–Gly–Phe–Met) were obtained from Bachem. Alanyl–methionine (Ala–Met), valyl–methionine (Val–Met), leucyl–methionine (Leu–Met), seryl–methionine (Ser–Met), prolyl–methionine (Pro–Met) and *S*-methylglutathione were purchased from Sigma. Histidyl–methionine (His–Met) and glycyl–methionyl–glycine (Gly–Met–Gly) were bought from Serva, and threonyl–methionine (Thr–Met) from Research Plus Inc. All compounds were of purest commercially available grade and used as received. Reagent grade NaOH and $HClO_4$ were added to the solutions for adjustment of pH. The solvent was 'Millipore'-filtered water.

Irradiations were carried out in the field of a 6000 Ci ^{60}Co-γ-source. The dose rate was measured by normal Fricke dosimetry (*i.e.* oxidation of $Fe^{2+} \longrightarrow Fe^{3+}$ in air saturated, acid aqueous solutions) and was about 1200 Gy h^{-1} (1 Gy = 1 J kg^{-1}). Details of the analytical procedure, experimental arrangements and the evaluation of data from HPIC experiments have already been described.[13,18]

Transient optical absorption spectra in aqueous solutions of methionine-containing peptides were measured by employing the radiation chemical technique of pulse radiolysis with time-resolved optical detection. The pulse experiments were performed with two different electron accelerators: Risø's 10 MeV HRC linear accelerator, and a 1.55 MeV Van de Graaff of the Hahn-Meitner Institute Berlin both providing short pulses (typically of 0.3–1 μs duration) of high energy electrons. The doses were in this case determined either with the ferrocyanide or thiocyanate dosimeter. Details of dosimetry measurements as well as all other aspects of the experimental pulse radiolysis set-up and the evaluation of data have been described elsewhere.[19,20] The typically applied dose per pulse was in the order of 5–10 Gy. This corresponds to a radical concentration of *ca.* (3–6) × 10^{-6} mol dm^{-3} for a radiation chemical yield of G = 6 (G denotes the number of species formed or converted per 100 eV absorbed energy; G = 1 corresponds to 0.1036 μmol per J absorbed energy in aqueous solutions; for practical purposes the G-unit rather than the SI-unit is used throughout the paper). A yield of 'OH radicals of this magnitude is generated, for example, in N_2O saturated (*ca.* 2 × 10^{-2} mol dm^{-3}) aqueous solutions generally employed for oxidation reactions. In such solutions all hydrated electrons, formed simultaneously with 'OH at about equal yield, are converted into 'OH *via* $e_{aq}^- + N_2O \longrightarrow {}^{\bullet}OH + OH^- + N_2$ so that the 'OH radicals account for 90% of all reactive primary species (the remaining 10% are H' atoms).

Carbon dioxide analysis was performed by means of high performance ion-chromatography (HPIC). Solutions were adjusted to pH 12, prior to analysis, by adding an appropriate amount of carbonate-free NaOH in order to convert CO_2 to its anionic forms. A DIONEX 2010i ion-chromatograph equipped with a 25 cm column HPICE-AS 1 in combination with a conductivity cell and water, pH 7, was used for separation and detection of HCO_3^-. The elution time of hydrogen carbonate ranged from 6.5–10 min depending on the individual column and the flow-rate. Calibration was accordingly done for each daily set of experiments. It was achieved by injecting known amounts of CO_2 gas (up to 200 mm^3 into 20 cm^3 solution) into unirradiated solutions at pH 12. The actual molarity was calculated using the ideal gas law and based on complete absorption of the CO_2 in the solution. G-values were calculated from the slopes of yield–dose plots.

The pK_a values of the carboxyl and amino function were obtained by pH-titration using 10^{-3} mol dm^{-3} solutions of the respective peptide and NaOH (1 mol dm^{-3}) or HCl (1 mol dm^{-3}). The actual pH-values were measured using a Knick digital pH-meter.

All experiments were carried out at room temperature. Error limits are estimated to ±10% for individual experiments. The actual error limits of the chromatographic yields and pulse radiolysis data are probably considerably lower due to

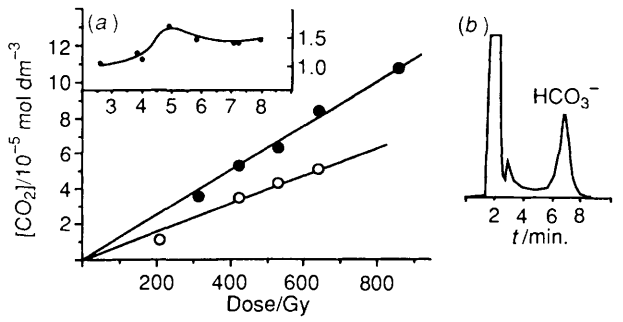


Fig. 1 CO_2 yield *vs.* radiation dose in N_2O-saturated solutions of 2×10^{-3} mol dm^{-3} peptides: Gly-L-Met (●), Gly-L-Met + 10^{-4} mol dm^{-3} ABTS (○); (*a*) pH-dependence of the CO_2-yield (expressed in *G*) in N_2O-saturated solutions of 10^{-3} mol dm^{-3} Gly-L-Met (*x*-axis: pH; *y*-axis: *G*); (*b*) ionchromatogram of an N_2O-saturated solution of 2×10^{-3} mol dm^{-3} Gly-L-Met irradiated at pH 5.6.

averaging over a least six individual data points for each experiment.

Results

CO_2 *Formation from Gly–Met.*—Oxidation of the most simple X-Met peptide, namely, Gly–Met, (for structure see Discussion section and Tables) by $^{\bullet}OH$ radicals in slightly acid and neutral solutions results in the formation of CO_2. A representative ion chromatogram referring to a γ-irradiated, N_2O-saturated solution of Gly–Met (2×10^{-3} mol dm^{-3}) at pH 5.6 is shown in the insert (*b*) in Fig. 1. The hydrogen carbonate peak is well separated from the others and thus easily quantified. The CO_2 concentration formed upon irradiation depends linearly on the absorbed dose (Fig. 1, full circles). From the slope of the corresponding straight line $G(CO_2) = 1.2$ was derived. This value is considerably lower than the yield of $^{\bullet}OH$ radicals (*G ca.* 4.7) which directly attack the sulphur atom and lead to decarboxylation in unsubstituted methionine at this pH.[5,10] (A quantitative evaluation on the yield of $^{\bullet}OH$ radicals attacking the methionine sulphur in all peptides studied is given in the Discussion section). $G(CO_2)$ hardly varies with the Gly–Met concentration. As can be seen from Table 1 it drops by *ca.* 10% in going from 5×10^{-4} to 4×10^{-3} mol dm^{-3} peptide.

The pH dependence of the $^{\bullet}OH$ induced decarboxylation is shown in insert (*a*) in Fig. 1 for solutions of Gly–Met (10^{-3} mol dm^{-3}). Starting from the low-pH side, $G(CO_2)$ is first seen to increase to a slight maximum (*G ca.* 1.7) at pH 4.8 before it finally attains a plateau (*G ca.* 1.4) between pH 5.6 and 8. The half-value of the initial rising portion of the curve is located at pH *ca.* 4 and close to the p*K* of the carboxyl group of this peptide (3.5). As will be discussed later the p*K* of the carboxyl group in the peptide is an important parameter controlling the decarboxylation.

CO_2 *Formation from Gly–Gly–Met and Enkephalins.*—Increase of the length of the peptide backbone, accomplished by addition of the glycine residue at the *N*-terminal of the Gly–Met dipeptide, is manifested in higher CO_2 yields as compared to Gly–Met as can be appreciated from the data in Table 1. There is practically no concentration dependence of the CO_2 yields.

Inclusion of a phenylalanine moiety, *i.e.* an aromatic ring as in Gly–Gly–Phe–Met (Des^1-Tyr-enkephaline) results in a significantly lower absolute CO_2 yield ($G = 0.48$). This is easily understood in terms of the well known high reactivity of the electrophilic $^{\bullet}OH$ radicals towards π-systems, leaving less $^{\bullet}OH$ for the decarboxylation route.

The presence of a tyrosine function in the enkephaline Tyr–Gly–Gly–Phe–Met reduces the absolute decarboxylation yields as compared to Gly–Gly–Phe–Met (*G ca.* 0.1 *vs.* 0.48). Tyrosine is also known to be an effective $^{\bullet}OH$ scavenger, and furthermore to transfer electrons to oxidized sulphur functions.[2,3,15,21]

Effect of Additives.— The CO_2 yields are not only a function of pH and peptide concentration but could also be influenced by other parameters. For example, addition of 10^{-4} mol dm^{-3} ABTS [2,2′-Azinobis-(ethylbenzthiazoline-6-sulphonate), known as a potent electron donor] to 2×10^{-3} mol dm^{-3} solutions of Gly–Met results in a *ca.* 35% decrease of $G(CO_2)$. This is documented in Fig. 1 (open circles) where the corresponding yield–dose plot exhibits a lower slope than the Gly–Met system alone. It should be noted that, although ABTS is a good $^{\bullet}OH$ scavenger itself[22] it is still the peptide which in these solutions scavenges the majority (≥95%) of the $^{\bullet}OH$ radicals; $k(^{\bullet}OH + \text{Gly–Met})$ is assumed to be equal to $k(^{\bullet}OH + \text{Met}) = 2.2 \times 10^{10}$ dm^3 mol^{-1} s^{-1}.[5] The result thus implies interference of the electron donor at a later stage of the reaction mechanism as will be considered in the Discussion.

Addition of cysteine (pK_{SH} 8.36)[23] at a pH where the neutral thiol form prevails (*e.g.* 2×10^{-4} mol dm^{-3} solution of cysteine added to solutions of 2×10^{-3} mol dm^{-3} Gly–Met at pH 5.2) also reduces the decarboxylation yield but not as much as ABTS. This is understandable as thiols are the comparatively weaker antioxidants.

Addition of an efficient proton acceptor (*e.g.* 10^{-3} mol dm^{-3} $H_2PO_4^-$/10^{-3} mol dm^{-3} HPO_4^{2-} added to pH 6.6 solutions of 2×10^{-3} mol dm^{-3} Gly–Met) similarly lowers the CO_2 yield. Proton acceptors are known to convert sulphur centred $R_2S^{\bullet+}$ type radical cations into an α-thioalkyl radical by removal of a proton.[24]

CO_2 *Formation from X-Met Peptides and* N*-Acetylmethionine* (*X = Ala, β-Ala, Val, Leu, Pro, Ser, Thr, His and γ-Glu*).—γ-Irradiation of N_2O-saturated, pH 5.6–6.5 solutions of X-Met peptides (10^{-3} mol dm^{-3}) with X = Ala, Val, Leu and Pro, respectively, results in CO_2 yields which decrease with the length and bulkiness of the alkyl side chain of the *N*-terminal amino acid X. The respective data are summarized in Table 2.

In order to study the influence of charge on the efficiency of decarboxylation the investigations were extended to His–Met, γ-Glu–Met and *N*-acetylmethionine (*N*-Ac–Met) (see Table 3). Depending on pH these peptides can bear an additional charge in the side chain[25] and thus differ, with respect to the overall charge, from the zwitterionic but overall neutral peptides described in the previous chapter.

Particularly high CO_2 yields are found upon oxidation of γ-Glu–Met, a peptide whose X-function (γ-Glu) is overall neutral (although zwitterionic) and which contains two carboxy groups, one on each amino acid moiety, as potential sites for CO_2 elimination. At pH 5.5 the CO_2 yield ($G = 4.55$) is practically identical with that measured from methionine itself, accounting for approximately 80% of all $^{\bullet}OH$ radicals available.

A lower but still very significant decarboxylation yield is obtained from *N*-Ac–Met (2.65) and β-Ala–Met (2.8) (Table 3). The overall charge of the first molecule is negative and resides on the carboxy function of the methionine. Going from *N*-Ac–Met to Gly–Met the CO_2 yield is essentially cut down again by a factor of two. Comparison with Gly–Met thus directly reveals the influence of a protonated amino group in the otherwise almost identical compound. However, in the case of γ-Glu–Met the explanation is not that simple as becomes apparent when comparing the CO_2 yields of γ-Glu–Met and *N*-Ac–Met, both peptides having a zero net charge after oxidation of the sulphur.

In the presence of a histidine moiety (His–Met) decarboxylation is drastically reduced. One reason is possible trapping of $^{\bullet}OH$ by addition to the double bonds and the nitrogen lone pairs. The difference between the pH 4.9 ($G = 0.46$) and pH 6.7

Table 1 Yields of $^{\bullet}OH$ radical-induced CO_2 formation (expressed in G) in N_2O-saturated solutions of various concentrations of methionine-containing peptides (pH 5.6)

Peptide	Formula	Concentration/ mol dm^{-3}	$G(CO_2)$
Gly–Met	H_3N^+–CH_2–CONH-Met[d]	5×10^{-4}	1.33
		1×10^{-3}	1.30
		2×10^{-3}	1.22
			0.80[a]
			1.01[b]
			0.93[c]
		4×10^{-3}	1.18
Gly–Gly–Met	H_3N^+–CH_2–CONH–CH_2–CONH-Met[d]	5×10^{-4}	1.39
		1×10^{-3}	1.46
		2×10^{-3}	1.33
			0.58[a]
			1.09[b]
			1.23[c]
		4×10^{-3}	1.37
Met–Gly	Met[e]-CONH–CH_2–CO_2^-	1×10^{-3}	<0.2
Met–Glu	Met[e]-CONH–CH(CO_2^-)–CH_2CH_2–CO_2^-	1×10^{-3}	<0.2
Met–Gly–Gly	Met[e]-CONH–CH_2–CONH–CH_2–CO_2^-	1×10^{-3}	<0.2
Gly–Met–Gly	H_3N^+–CH_2–CONH-Met[f]-CONH–CH_2–CO_2^-	1×10^{-3}	<0.1

[a] With 10^{-4} mol dm^{-3} ABTS. [b] With 1:1 mixture of $H_2PO_4^-/HPO_4^{2-}$ at 10^{-3} mol dm^{-3} concentration. [c] With 2×10^{-4} mol dm^{-3} cysteine. [d] Met denotes: –CH(CH_2CH_2–S–CH_3)–CO_2^-. [e] Met denotes: H_3N^+–CH(CH_2CH_2–S–CH_3)–. [f] Met denotes: –CH(CH_2CH_2–S–CH_3)–.

Table 2 Yields of $^{\bullet}OH$ radical induced CO_2 formation (expressed in G), and efficiency of decarboxylation normalized to the yield of the OH adduct to sulphur (expressed as $f = G(CO_2)_{exp}/G$[S-oxid. in Met]) in N_2O-saturated solutions of 10^{-3} mol dm^{-3} X–Met peptides (X = Gly, Ala, Val, Leu, Pro, Thr) at pH 3.7, 5.9 and 8.0 (for pK-values see footnote *b*, pK_I for CO_2H/CO^-, pK_{II} for NH_3^+/NH_2)

		$G(CO_2)_{exp}$			$G(CO_2)_{exp}/G$(S-oxid. in Met)		
Peptide	Formula	pH 3.7	5.9	8.0	3.7	5.9	8.0
Gly–Met	H_3N^+–CH_2-CONH–Met[a]	1.16	1.46	1.46	0.25	0.31	0.31
Ala–Met	H_3N^+–CH(–CH_3)–CONH–Met[a]	1.03	0.98	1.22	0.22	0.21	0.26
Val–Met	H_3N^+–CH(–CH(CH_3)(CH_3))–CONH–Met[a]	0.83	0.64	1.03	0.20	0.16	0.25
Leu–Met	H_3N^+–CH(–CH_2–CH(CH_3)(CH_3))–CONH–Met[a]	0.77	0.61	0.97	0.19	0.15	0.24
Pro–Met	H_2N^+(–CH_2–CH_2–CH_2–)–CH–CONH–Met[a]	—	0.20	—	—	0.04	—
Thr–Met	H_3N^+–CH(–CHOH–CH_3)–CONH–Met[a]	—	1.30	1.40	—	0.30	0.32

[a] Met denotes: –CH(CH_2CH_2–S–CH_3)–CO_2^-. [b] Gly–Met (pK_I 3.5, pK_{II} 7.95), Ala–Met (3.4, 7.50), Val–Met (3.5, 7.62), Leu–Met (3.3, 7.75).

(G *ca.* 0.3) can be explained by the pK = 6.0 of the imidazole nitrogen.[23] The probability of the electrophilic $^{\bullet}OH$ radical reacting with the imidazole becomes much higher if this is not protonated.

CO_2 *Formation from Met–Gly, Met–Gly–Gly and Gly–Met–Gly.*—Changing the order of the amino acids in the peptides may lead to very different results with respect to the decarboxylation. γ-Irradiation of N_2O-saturated 10^{-3} mol dm^{-3} solutions of Met–Gly, Met–Gly–Gly or Gly–Met–Gly, *i.e.* peptides which do not possess a *C*-terminal methionine residue, did not yield any remarkable amounts of CO_2. This identifies the

Table 3 Yields of 'OH radical-induced CO_2 formation (expressed in G), and efficiency of decarboxylation normalized to the yield of the OH adduct to sulphur (expressed as $f = G(CO_2)_{exp}/G$[S-oxid. in Met]) in N_2O-saturated solutions of 10^{-3} mol dm^{-3} X-Met peptides (X = Ser, His, β-Ala, N-Ac, γ-Glu) at pH 5.6

Peptide	Formula	$G(CO_2)_{exp}$	$G(CO_2)_{exp}/G$(S-oxid. in Met)
Ser–Met	H_3N^+–CH(CH$_2$OH)–CONH–Met[a]	0.50	0.11
His–Met	H_3N^+–CH(CH$_2$–C(=CH–$^+$NH–CH=N)–H)–CONH–Met[a,b]	0.45 (pH 4.9) 0.30 (pH 6.7)	0.11 0.10
β-Ala–Met	H_3N^+–CH_2CH_2–CONH–Met[a,c]	2.80	0.61
N-Ac–Met	CH_3–CONH–Met[a]	2.65	0.58
γ-Glu–Met	H_3N^+–CH(CO_2^-)–$(CH_2)_2$–CONH–Met[a,d]	4.55	1.00

[a] Met denotes: –CH(CH_2CH_2–S–CH_3)–CO_2^-. [b] pK = 6.0 (imidazole nitrogen in side chain). [c] pK_I = 3.5, pK_{II} = 9.2. [d] pK_I = 2.95, pK_{II} = 3.75, pK_{III} (NH_3^+/NH_2) = 9.15.

importance of the sulphur function and the free carboxylate which apparently must both be available in the *C*-terminal amino acid moiety in order to facilitate decarboxylation.

Pulse Radiolysis.—In a previous pulse radiolysis investigation on the 'OH induced oxidation of methionine and derivatives[10] it had been noted that stabilization of an S∴S bonded, dimeric radical cation and generation of an α-amino radical, which results from decarboxylation, seemed to be complementary processes. In order to check whether such a simple competition also applies for methionine-containing peptides transient absorption spectra were recorded in pulse irradiated, N_2O-saturated, aqueous solutions of X–Met peptides. γ-Glu-Met and Pro–Met would appear to be particularly suited for such investigations since their decarboxylation yields in slightly acid environment (*e.g.* pH 5.6) represent two extreme cases (see Tables 2 and 3).

Figs. 2(*a*) and 2(*b*) show the spectra recorded immediately after the (*ca.* 1 μs) pulse from solutions of concentration 10^{-3} mol dm^{-3}, and at pH 1 and pH 5.6, respectively, of γ-Glu-Met and Pro-Met. Under the very acidic conditions at pH 1 both peptides yield practically identical transient absorptions (note different scale on *y*-axis) with two maxima. The respective peaks in the visible at λ *ca.* 480 nm are typical for S∴S bonded, dimeric radical cations. In analogy to numerous examples[2-10,15,16,24] they are assigned to the corresponding γ-Glu–Met and Pro–Met species. The UV band at λ *ca.* 290 nm is characteristic for α-thioalkyl radicals[5,26] (...–'CH–S–...) which result from deprotonation of the $R_2S^{\cdot+}$ type radical cation[24] or direct H-atom abstraction by 'OH radicals.

The most remarkable aspect of the spectra at pH 5.6 is the lack of the λ = 480 nm absorption in both systems which discards a simple correlation between the formation of the S∴S bonded dimeric radical cation and the decarboxylation process. More revealing are the UV spectra. In case of γ-Glu–Met the spectrum resembles that of the α-amino radical formed as a result of decarboxylation of the S∴N bonded intermediate from methionine, **1**, and can thus be attributed to

H_3N^+–CH(CO_2^-)–$(CH_2)_2$–CO–NH–$\dot{C}$H–$(CH_2)_2$–S–CH_3

and/or

H_2N–$\dot{C}$H–$(CH_2)_2$–CO–NH–CH(CO_2H)–$(CH_2)_2$–S–CH_2

A different result is obtained from Pro–Met. The UV band is qualitatively identical with that at pH 1 and therefore attributable to the α-thioalkyl radical, but it assumes twice that yield at pH 5.6.

The pulse radiolysis results for Pro–Met are corroborated by those for Gly–Met–Gly. For both peptides no decarboxylation occurs and accordingly the only transients observable are the $(>S\therefore S<)^+$ type radical cation and the α-alkyl radical, as indicated from the λ = 480 and 290 nm absorptions in Fig. 2(*c*). However, while the three-electron bonded species is not formed at all from Pro–Met at pH 5.6, a significant yield of this dimer radical cation is stabilized from Gly–Met–Gly under the same conditions. The same is true for other peptides such as Gly–Met, Gly–Gly–Met and *N*-Ac–Met. The respective yields of the $(>S\therefore S<)^+$ absorption at λ = 480 nm (in terms of G) in pH 5.6 solutions are listed in Table 4. Comparison with the CO_2 yield (also listed in Table 4) once more demonstrates that S∴S bond formation and decarboxylation are not complementary in a simple competitive two-way mechanism. In fact, for these peptides higher $(>S\therefore S<)^+$ yields are accompanied by higher CO_2 yields.

The $(>S\therefore S<)^+$ yields are calculated on the basis of the extinction coefficients which were derived from the pH 1 experiments under the assumption that *ca.* 80% of the initial 'OH radicals react with the Met-sulphur and quantitatively shows up as this three-electron bonded radical cation as for simple methionine.[5] The ε-values derived this way are also included in Table 4. They are of the expected order of magnitude[5,6] but should be associated with relatively large error limits (±30%) owing to the as yet unknown equilibrium constants for the $(>S\therefore S<)^+$ stabilization according to eqn. (1) and rate constants for the $>S^{\cdot+}$ deprotonation. As the actual $(>S\therefore S<)^+$ yields from the peptides are probably somewhat lower than for free methionine, the presently evaluated ε-values would represent only lower limits. The value for *N*-Ac–Met, on the other hand, is likely to be too high considering that the sum of $(>S\therefore S<)^+$ and CO_2 yields derived on its basis at pH 5.6 amounts to almost 100% (rather than 80%) of the total amount of 'OH radicals.

Finally, it should be noted that pulse radiolysis of all peptides investigated in this study did not provide evidence for stabilization of an intramolecular sulphur–carboxylate intermediate such as, for example, the one depicted in structure **2**

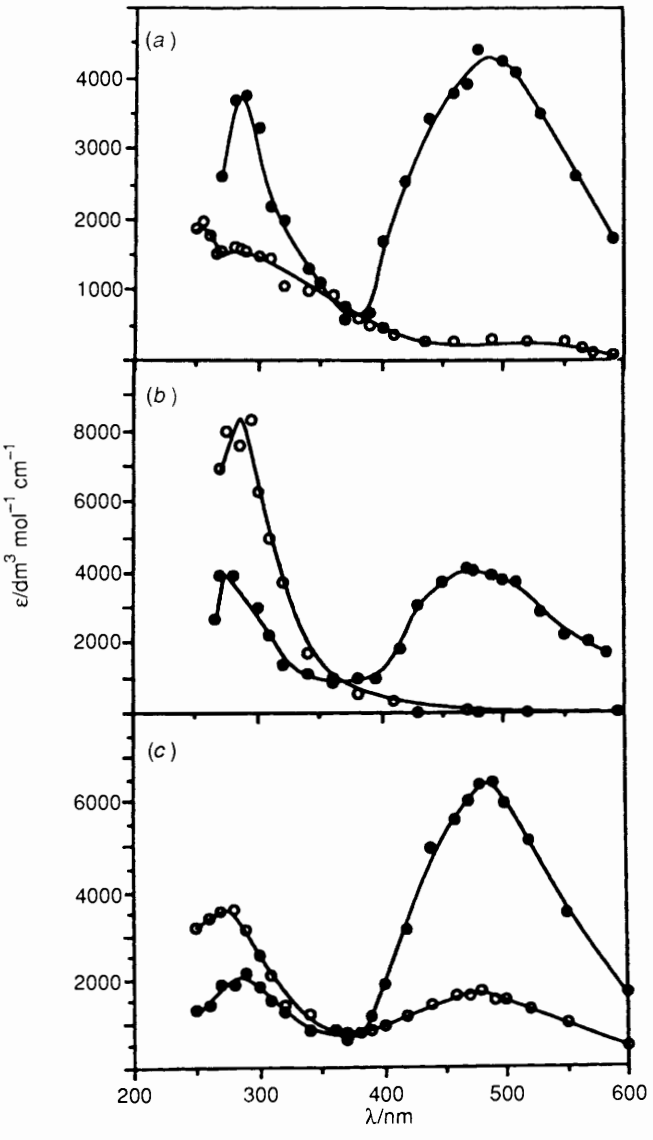


Fig. 2 (*a*) Transient absorption spectra recorded *ca.* 1 μs after the electron pulse in irradiated N_2O-saturated solutions of 10^{-3} mol dm^{-3} γ-Glu-L-Met at pH 1 (●), and pH 5.6 (○); (*b*) transient absorption spectra recorded *ca.* 1 μs after the electron pulse in irradiated N_2O-saturated solutions of 10^{-3} mol dm^{-3} L-Pro-L-Met at pH 1 (●), and pH 5.6 (○); (*c*) transient absorption spectra recorded *ca.* 1 μs after the electron pulse in irradiated N_2O-saturated aqueous solutions of 10^{-3} mol dm^{-3} Gly–Met–Gly at pH 1 (●) and pH 5.6 (○)

from the norbornane derivative amino acid [11] or from *S*-methyl cysteine.[7]

Discussion

Yield of Sulphur Oxidation in the Methionine Moiety.—Our results on the ·OH induced oxidation of methionine-containing peptides have shown that decarboxylation requires the presence of a *C*-terminal methionine moiety and occurs particularly efficiently when both the sulphide function and a free carboxy group are located in the same *C*-terminal peptide unit. Separation of these two key functionalities into different amino acids, as in *e.g.* Met–Gly, Met–Glu, Met–Gly–Gly and Gly–Met–Gly practically prevents decarboxylation. These findings can be rationalized in terms of an *intra*molecular mechanism based on an interaction between the oxidized sulphur function and the carboxy group. Structurally this is facilitated by the possibility of arranging sterically favourable six-membered ring configurations with respect to sulphur and any of the two carboxy oxygen atoms.

Table 4 Extinction coefficients and yields of intermolecular S∴S-bonded radical cations, and yields of CO_2 measured in solutions of X–Met peptides

Peptide	$\varepsilon_{480}(>S\therefore S<)^{+}$ [c]	$G[(>S\therefore S<)^{+}]$ pH 5.6	$G[CO_2]$ pH 5.6
Gly–Met[a]	5900	2.3	1.30
Gly–Gly–Met[a]	6100	2.7	1.46
Gly–Met–Gly[a]	6450	1.2	≤0.1
N-Ac–Met[b]	9600	3.1	2.7
Pro–Met[a]	4100[d]	0.0	≤0.2
γ-Glu–Met[a]	4400[d]	0.0	4.55

[a] Concentration 1×10^{-3} mol dm^{-3}. [b] Concentration 2×10^{-3} mol dm^{-3}, taken from ref. 5. [c] Estimated from results at pH 1. [d] Values probably too low because of fast deprotonation at pH 1.

The primary target of oxidative attack by the ·OH radical is considered to be the methionine–sulphur. This unit exhibits the lowest ionization energies and highest rate constants for oxidation in comparable sulphide compounds[27] including methionine itself. In case of methionine it has been found that the combined yield of observable $(>S\therefore S<)^{+}$ type intermediates and CO_2 which unambiguously originate from direct sulphur oxidation, accounts for 80% of all ·OH radicals attacking this amino acid[5] (and even for the remainder this pathway has to be taken into consideration). In terms of radiation chemical yield units this figure corresponds to *G ca.* 4.8 in N_2O-saturated solutions, *i.e.* is close to the maximum CO_2 yield now obtained from γ-Glu-Met, the peptide exhibiting the highest decarboxylation efficiency. This suggests that the presence of the additional glutamic acid unit has little, if any, effect on the yield of primary oxidation. However, this is not surprising considering the relative reactivities of ·OH radicals towards methionine (*k ca.* 2.2×10^{10} dm^3 mol^{-1} s^{-1})[5] and glutamic acid (*k ca.* 5×10^{8} dm^3 mol^{-1} s^{-1}), respectively, according to which just 2% of the ·OH would be expected to react with the glutamic acid moiety. A similar argument applies to most of the other X–Met peptides. Rate constants between 7×10^{7} and 6×10^{8} dm^3 mol^{-1} s^{-1} have been reported for the reaction of ·OH and the zwitterionic forms of X = Ala, Gly, Pro, Ser, Thr and Val.[27] Slightly lower values apply for the corresponding reaction with the acid form which, from the point of view of charge, resembles the peptide even more. Calculations on this basis reveals that less than 7.5% of ·OH reacts with X. Higher yields are only to be expected for Leu–Met and His–Met, for which 20% and 38% of the ·OH, respectively, would not react with the Met-unit ($k_{Leu+\cdot OH} = 1.7 \times 10^{9}$ dm^3 mol^{-1} s^{-1}, and $k_{His+\cdot OH} = 5 \times 10^{9}$ dm^3 mol^{-1} s^{-1}).[27]

For better assessment of the peptide decarboxylation yields from the various X–Met we have calculated the respective yields of remaining ·OH attack at the Met–sulphur on the basis of the above mentioned rate constants, taking the isolated methionine value of $G = 4.8$ as reference (Tables 2 and 3). The ratios between the actually measured CO_2 yields and these figures denote, in turn, the fractions of ·OH attack at sulphur which ultimately result in decarboxylation (Tables 2 and 3). Although relatively large error limits have to be allowed for this procedure, it is quite clear that primary oxidation at sulphur is only a prerequisite for decarboxylation (as had been established in the case of methionine).[5] As will be discussed after the establishment of the reaction mechanism it appears that factors like (i) the electronic nature of the side chain R in the Met-substituents X, (ii) the overall charge of the peptide molecule, (iii) structural parameters such as the distance between positively charged centres ($-NH_3^{+}$ and $>S^{\cdot+}$), and (iv) the

influence of all this on the kinetics of the possible reactions of the key intermediate, the $>S^{\bullet+}$ radical cation, play a decisive role.

Reaction Mechanism.—Addition of a proton to the hydroxy adduct **3** yields the more stable radical cation $(>S\therefore OH_2)^+$, **4**, which can be viewed as a complex between the molecular radical cation $>S^{\bullet+}$ and a water molecule.[28–30] For convenience this species is generally referred to as $>S^{\bullet+}$ in the following. Based on the results from previous studies with methionine,[5] *S*-methyl cysteine,[7,12] simple organic sulphides[4,6,7,24] and sterically rigid norbornane derivatives[11,31] it is possible to propose a reaction mechanism for the reactions initiated by $^{\bullet}OH$ attack at the methionine sulphur atom. This is outlined in reactions (2)–(7) and Scheme 1. The initial step is $^{\bullet}OH$ addition to the sulphur, [eqn. (2)].

$$^{\bullet}OH + >S \longrightarrow \underset{\mathbf{3}}{>S\therefore OH} \qquad (2)$$

A displacement reaction involving a second molecule leads to the dimeric $(>S\therefore S<)^+$ centred radical cation **5** as shown in Scheme 1.

$$3 + H^+ \longrightarrow \underset{\mathbf{4}}{(>S\therefore OH_2)^+}$$

$$+ >S \;\downarrow\uparrow\; -H_2O$$

$$\underset{\mathbf{5}}{(>S\therefore S<)^+}$$

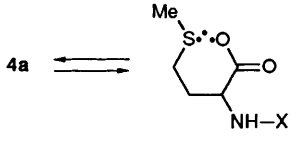
Scheme 1

This is the species which absorbs at *ca.* 480 nm (see Fig. 2). It exists in equilibrium with the molecular radical cation $>S^{\bullet+}$ **4** with the relative amounts being controlled by the peptide concentration (equilibrium constants may be expected in the 10^3–10^5 $dm^3\ mol^{-1}$ range in favour of the three-electron bonded species).[24] A more detailed structure of **4** is shown in **4a**.

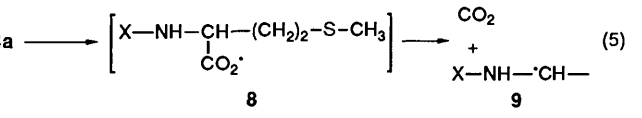
$$\underset{\mathbf{4a}}{\begin{array}{l} X\text{–}NH\text{–}CH\text{–}(CH_2)_2\text{–}\overset{\bullet+}{S}\text{–}CH_3 \\ \quad\;\; | \\ \quad COO^- \end{array}}$$

Besides equilibration to **5** the sulphur-centred radical cation **4a** has three other principal possibilities to react, as summarized in eqns. (3)–(5), namely deprotonation at C α to S, bond formation with the carboxy function *via* ring closure, and electron transfer coupled with decarboxylation.

$$\mathbf{4a} \longrightarrow H^+ + \underset{\mathbf{6}}{>C\text{–}S\text{–}} \qquad (3)$$



(4)



(5)

Deprotonation of **4a**, occurring at the carbon atom α to sulphur, leads to the α-thioalkyl radical **6** [eqn. (3)] which exhibits moderate reducing properties[4] and an optical absorption with λ_{max} *ca.* 290 nm (see Fig. 2). Alternatively, a proton could be lost from the protonated amino group in the X-moiety. The driving force for this process could be the coulombic interaction between the two positive charges in **4a** (at $>S^{\bullet+}$ and $-NH_3^+$) resulting in a shift of the p*K* to lower values for this amino group in the oxidized as compared to the unoxidized peptide. (Interaction of the oxidized sulphur with the nitrogen in the peptide bond followed by decarboxylation, as in methionine, can be excluded).*

The ring closure reaction (4) is considered to be reversible as is the $(>S\therefore S<)^+$ formation in Scheme 1. Species **7** is characterized by stabilization of the oxidized sulphur through bond formation with the methionine–carboxyl group. This *intra*molecular sulphur–oxygen interaction species is facilitated by a sterically favourable ring formation and resembles features of neighbouring group participation established in the oxidation of the norbornane derivative of methionine[11] (see Introduction). Such species typically absorb in the λ 340–400 nm range.[7,11,31] In our present investigation (*e.g.* see Fig. 2) no evidence has been found for **7**. This does, however, not exclude this possibility since sulphur–oxygen interactions of this kind are very weak in flexible molecules[7] and furthermore exist in equilibrium with the $(>S\therefore S<)^+$ bonded species **5** [eqn. (6)].[32]

$$7 + >S \rightleftharpoons 5 \qquad (6)$$

The chemical fate of **7** is thus linked to the **4** ⇌ **5** equilibrium and consequently to all the reactions of **4** and **5**. In addition, species **7** suffers fast irreversible decay through deprotonation [eqn. (7)].[11]

$$7 \longrightarrow 6 + H^+ \qquad (7)$$

The mechanism leading to decarboxylation appears to be the one which involves electron transfer [eqn. (5)] from the carboxylate function. Such a mechanism, corroborating the well known Kolbe reaction in electrochemistry and even more so the pseudo-Kolbe reaction,† has been demonstrated to operate in the oxidation of the norbornane derivative of methionine.[11] The α-amino type radical **9**, $X\text{–}NH\text{–}^{\bullet}CH\text{–}(CH_2)_2\text{–}S\text{–}CH_3$, exhibits a UV absorption as exemplified in the pH 5.6 spectrum in Fig. 2(*a*) (steady rise towards lower wavelengths without noticeable maximum). It is specifically noted that electron transfer does not seem to take place within the sulphur–carboxylate bond of **7**.[11] Deprotonation of the carboxy group is a necessary prerequisite for the decarboxylation mechanism. It explains the decrease in CO_2 yield and the corresponding increase in stabilization of **5** towards acid pH.

Deprotonation.—The overall reaction mechanism reveals two important irreversible channels which for pH > p*K*($-CO_2H$) seemingly decide on the decarboxylation yield, namely, electron transfer from the carboxy group to the sulphur-centred radical cation $>S^{\bullet+}$ [eqn. (5)] ultimately leading to CO_2, and

* Interaction between the oxidized sulphur and the nitrogen in the peptide bond is unlikely for coulombic reasons. Owing to resonance with the neighbouring carbonyl the peptide nitrogen should carry enough positive charge [$-NH\text{–}CO- \longleftrightarrow -NH^+\text{=}C(O^-)-$] to prevent efficient approach of the $>S^{\bullet+}$ centre. Also, if such an interaction took place (and induced decarboxylation, as in free methionine) significant CO_2 yields would have been observed, for example, in the case of Gly–Met–Gly, contrary to the experimental result which showed practically no decarboxylation.

† Pseudo-Kolbe reactions involve oxidation of a site remote to carboxylate followed by electron transfer and decarboxylation. See for example: P. G. Gassman and B. L. Fox, *J. Chem. Soc., Chem. Commun.*, 1967, **32**, 480; *Organic Electrochemistry*, eds. M. M. Baizer and H. Lund, 2nd edn., Marcel Dekker, New York, 1983, pp. 443, 455 and 574.

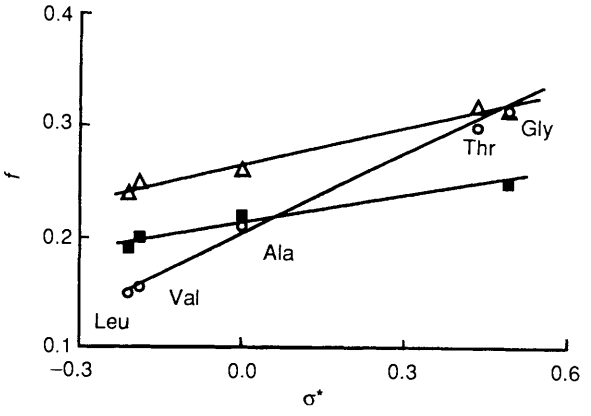


Fig. 3 Decarboxylation efficiency f (see text) *vs.* Taft inductive parameters (σ*) of the substituents at the α-carbon of the *N*-terminal amino acid in X-Met peptides at pH 3.7 (■), 5.9 (○) and 8.0 (△)

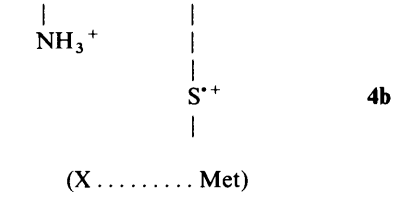
deprotonation at $>S^{\bullet+}$ not leading to CO_2 [eqn. (3)]. In the following it will be discussed how these two competing reaction channels are affected by structural parameters, charges, the thermodynamics of the various equilibria involved or additives to the solution. For all our considerations it should further be remembered that (although not specifically shown in our graphical structures) any protonated amino group of X may interact with any carboxylate to form zwitterionic structures with a certain degree of stabilization energy.

Deprotonation reactions such as **4a** ⟶ **6** [eqn. (3)] can generally be accelerated by addition of proton acceptors.[24] This increases the effective rate of deprotonation and, in turn, reduces the probability of electron transfer (**4a** ⟶ **8**) [eqn. (5)] and subsequent decarboxylation. The observed decrease in CO_2 upon addition of phosphate (Table 1) would thus find a simple explanation.

The results listed in Table 2 indicate a strong dependence of the CO_2 formation on the substituent R in the X-component of the peptides. The observed trend parallels the inductive properties of R, with the highest CO_2 yield being formed if R = H (X = Gly) and the lowest if R = $CH_2CH(CH_3)_2$ (X = Leu). The Taft plots shown in Fig. 3 in terms of the efficiency of decarboxylation ($f = G(CO_2)_{exp}/G$[S-oxid. in Met] *vs.* Taft's inductive σ* parameters[33] exhibit straight lines with the highest slope at pH 5.9.

$$f(CO_2) = 0.2 + 0.25\,\sigma^*$$

This dependence can be understood by the mutual influence of the two positive charges in **4b** at sulphur and the terminal amino group in X.



4b

The pK_a values of the protonated terminal amino groups ($-NH_3^+$) in the unoxidized peptides are in the range 7–8 (see footnotes in Table 2). In the respective oxidized forms **4b**, however, they should be shifted to lower values owing to the influence of the second positive charge located at sulphur. It seems not unreasonable to assume pK_as in the 4–7 range. It is further known that the basicity of an amino function is directly related to the electron donating properties of adjacent substituents[34] and should, therefore, be affected by electron release from R. The results of Table 2 used for the Taft plot in Fig. 3 can be explained (at least qualitatively) if the pK_as of the amino functions in oxidized peptides are close to pH = 5.9. In this case increasing donation of electron density would result in a relatively large change of the $[-NH_3^+]/[-NH_2]$ ratio in favour of the protonated form. It is reasonable to assume that the coulombic interaction of the two positive charges in **4b** facilitates deprotonation at the α-position to the oxidized sulphur site, *i.e.* to enhance the overall rate constant of reaction (3). This, in turn, would reduce the probability for electron transfer and subsequent decarboxylation. Accordingly, the highest CO_2 yield is obtained for Gly–Met, the peptide with the lowest $[-NH_3^+]/[-NH_2]$ ratio.

These effects should be less pronounced at pH values which differ significantly from the respective pK_a values of the oxidized peptides, because the changes in the $[-NH_3^+]/[-NH_2]$ ratio then become increasingly negligible. A quantitative experimental verification is shown for pHs 3.7 and 8.0 with the respective slopes being around 0.10, *i.e.* much lower than at pH 5.9.

The Taft value for the threonine substituent [R = CH(OH)-CH_3] had to be estimated to σ* *ca.* 0.40–0.45†; taking this value the CO_2 yield of 30% falls right on the straight line of the Taft plot. A much lower yield than expected is, however, obtained for Ser–Met (R = CH_2OH, σ* = 0.53)[33] for which only 11% relative CO_2 yield has been measured. We have no conclusive explanation for this. A direct influence of the hydroxy group on the electron transfer can probably be excluded. The low CO_2 yield from Ser–Met would therefore reflect a particularly enhanced rate of deprotonation at $>S^{\bullet+}$. In the light of the above discussion this would, in turn, imply a significantly increased basicity of the neighbouring amino group which is perhaps achieved through interaction with the terminal hydroxy group. Another possibility might be an association of the hydroxy group with $>S^{\bullet+}$ to yield a sulphur–oxygen linked intermediate. Such structures are known to exist (although observable only under sterically controlled conditions)[28,35] and to decay by deprotonation.

The fact that secondary amino groups generally exhibit a much higher basicity than primary ones[34] would satisfactorily explain the very low CO_2 yield from Pro–Met (corroborated by the high yield of **6**).

A high CO_2 yield is observed in case of β-Ala–Met (2.8) compared to Ala–Met (1.0) (Tables 2 and 3). There is no explanation as yet for this result. Structurally, both peptides differ in location of the amino function with respect to the peptide bond resulting in a significant shift of the p*K*s in the non-oxidized molecule (9.2 for β-Ala–Met and 7.5 for Ala–Met). Interestingly the decarboxylation yield of β-Ala–Met is close to the yield of *N*-Ac–Met which also lacks an amino group in the α-position to the peptide bond.

The influence of overall charge on the deprotonation at $>S^{\bullet+}$ also becomes apparent in the results from γ-Glu-Met and *N*-Ac–Met. γ-Glu-Met contains an additional carboxy group in the glutamic acid moiety which at pH 5.6 is dissociated and able to inactivate the protonated amino group through zwitterionic interaction. Consequently there is no charge-induced acceleration of the deprotonation at $>S^{\bullet+}$, as in the case of *N*-Ac-Met. Both exhibit a higher decarboxylation efficiency compared to peptides which carry an overall positive net charge after oxidation. The fact that $G(CO_2)$ from γ-Glu–Met is still higher than from *N*-Ac–Met reflects an activation which the *N*-

† The Taft parameter σ* of C_2H_5 is *ca.* 0.1 units more negative than that of CH_3. A similar difference is assumed between CH_3CHOH and CH_2OH in Thr–Met and Ser–Met, respectively. As the latter is *ca.* 0.53, the former is estimated to be *ca.* 0.43.

terminal α-carboxyl group receives by the free amino group in γ-Glu–Met. Thus an additional mechanism operates *via* oxidation of the amino function and subsequent decarboxylation as observed for unsubstituted methionine.[5] This is also supported by high decarboxylation yields during oxidation of *S*-methylglutathione. Details on this will be reported separately.[36]

His–Met, on the other hand, contains two nitrogens in the histidine moiety. In very acidic solutions both will be protonated and the combined influence of two positive charges would leave little chance for the $>S^{\bullet+}$ to undergo electron transfer (and subsequent decarboxylation) in competition with deprotonation. However, this probably does not apply for the conditions of our experiments at pH 4.9 and 6.7 because the p*K* of the imidazole nitrogen in the oxidized peptide should be shifted to values considerably lower than p*K* 6.0 in the unoxidized form. The actual fraction of protonation may therefore still be very small at pH 4.9. The low CO_2 yields are reasonably explained by the high probability of $^{\bullet}OH$ radicals to add to the double bonds in the His-moiety. This route would be favoured if the imidazole nitrogen were not protonated in the unoxidized molecule which is in accord with the experimental finding of comparatively lower CO_2 yields at higher pH.

The distance argument for charge interaction becomes apparent in the CO_2 yields from Gly–Gly–Met as opposed to Gly–Met. AMBER-structure calculations indicate a distance of 3.61 Å between the terminal $-NH_3^+$ group and sulphur in the non-oxidized dipeptide and 4.72 Å in the tripeptide.* The coulombic interaction should therefore be less pronounced in the tripeptide. This, in turn, reduces the probability of deprotonation at $>S^{\bullet+}$, and thus indirectly enhances the yield of electron transfer and subsequent decarboxylation.

In the instance of the two enkephalines investigated the influence of yet another parameter arises. The relative decarboxylation yields (experimentally found CO_2 divided by the calculated yield of $^{\bullet}OH$ attack at sulphur; see first part of the Discussion) amounts to 0.14 for Gly–Gly–Phe–Met but only 0.04 for Tyr–Gly–Gly–Phe–Met. This difference is attributed to the possibility of intramolecular electron transfer to $>S^{\bullet+}$ from the tyrosine[15,21] which competes with the oxidation of the carboxylate function [eqn. (5)]. Competitive electron transfer can also be achieved intermolecularly as is evidenced by the reduction in CO_2 yield upon addition of ABTS to the peptide solutions.

An earlier publication[4] considered the formation of the **6**-type α-thioalkyl radicals not only *via* deprotonation of $>S^{\bullet+}$ [eqn. (3)] but possibly also *via* H_2O elimination from its precursor, the $>S^{\therefore}OH$-type adduct radical. In the present peptide systems this latter process cannot be of great significance since deprotonation from the neutral $>S^{\therefore}OH$ is not expected to be influenced by 'long range' coulombic interaction. Direct elimination of a proton has to be considered, however, for the sulphur–carboxylate interaction species **7**. In addition to possible ligand exchange [eqn. (6)] the only other reaction it seems to suffer is irreversible deprotonation to yield the α-thioalkyl radical **6** [eqn. (7)].

* Using the AMBER program the structures of the Gly–Met and the Gly–Gly–Met peptides have been approximated. The peptide bonds were all assumed to adopt a *trans* conformation. The rotational states described by the torsional angles Φ_i, ψ_i and χ_{ij} were adjusted in such a manner so as to obtain the minimum permitted distance between NH_3 and the sulphur atom in the Gly–Met peptide. The conformation of the *N*-terminal glycine residue in the Gly–Gly–Met peptide was described by similar torsional angles as for glycine in the Gly–Met peptide. The structures thus generated were subjected to the minimalization energy procedure. The distance of interest was calculated to be 3.61 Å in Gly–Met and 4.72 Å in Gly–Gly–Met.

Conclusion

The present investigation on the decarboxylation of methionine-containing peptides has demonstrated that this process can be initiated through intramolecular, presumably outer-sphere, electron-transfer from the free carboxy group to the oxidized sulphur. This finding confirms the conclusion drawn from a separate study with a singular, structurally well defined and sterically very rigid amino acid,[11] allowing the generalization of this concept. Decarboxylation initiated through electron transfer to a positively charged radical site, $>S^{\bullet+}$, has to compete with deprotonation at the α-carbon next to sulphur. The relative probabilities of the various possible reactions depend significantly on the number, location and interaction of charges in the molecule which affect in particular the deprotonation pathway. Even long range coulombic interactions and the electronic influence of side-chain substituents influence the yields of peptide decarboxylation.

Acknowledgements

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