DOI: 10.1002/chem.200600828

Reactions of Hydrogen Atoms with Met-Enkephalin and Related Peptides

Olivier Mozziconacci,[b] Krzysztof Bobrowski,[b] Carla Ferreri,[a] and Chryssostomos Chatgilialoglu*[a]

Abstract: The reactions of hydrogen atoms with enkephalins and related peptides have been investigated by radiolytic methods in aqueous solutions and lipid vesicle suspensions. Pulse radiolysis experiments indicate that methionine residue (Met) is the main target. In Met-enkephalin (Tyr-Gly-Gly-Phe-Met) the attack of the hydrogen atom occurs to about 50% on Met with formation of methanethiyl radical. The remaining percentage is divided roughly evenly between Tyr and Phe. With Leu-enkephalin (Tyr-Gly-Gly-Phe-Leu) the site of attack is limited to Tyr and Phe. Using a peptide–liposome (that is, 1-palmitoyl-2-oleoyl phosphatidylcholine vesicles) model, the *cis*–*trans* isomerization of phospholipids could be detected due to the catalytic action of thiyl radicals. The radiation chemical yields of the H$^{\bullet}$ and, consequently of $CH_3S^{\bullet}$ radical, was modulated by the experimental conditions and the nature of peptide. Large amounts of *trans* lipids observed in phosphate buffer vesicle suspensions indicated the efficient role of double-bond isomerization as marker of Met-containing peptide damage.

Keywords: methionine damage · peptides · pulse radiolysis · radicals · radiolysis

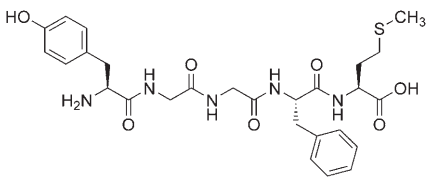
Introduction

Protein damage caused by free radicals makes part of the etiology of several diseases and the aging process. The most studied intermediates known to cause this damage are reactive oxygen species (ROS) and in particular, the $^{\bullet}$OH radicals.[1] The reductive radical stress has been considered much less,[2] although the first report by Stein and co-workers appeared in the sixties on proteins in dilute aqueous solutions.[3] The study examined the action of hydrogen atoms with bovine pancreatic ribonuclease (RNase A) and concluded that there were specific sites of attack to the sulfur-containing residues of the sequence. The observation of the H$^{\bullet}$ attack on methionine residues (Met) came from continuous radiolysis studies on single amino acid or Met-containing dipeptides, and also evidenced that the prime amino acid product was α-aminobutyric acid.[4,5] Recently, this reactivity has been placed in the context of a biological damage, since it can constitute the molecular basis of tandem protein–lipid damage.[6,7] Using a biomimetic system composed of Met-containing proteins, such as RNase A[6] or an amyloid β-peptide,[7] in the presence of liposomes made of unsaturated phospholipids, the protein damage caused by H$^{\bullet}$ to Met residues has been coupled with the formation of diffusible thiyl radicals. These species are able to migrate from the aqueous to the membrane compartment and cause damage to lipids, effecting the geometrical isomerization from *cis* to *trans* of the double bonds.

To our knowledge the role of H$^{\bullet}$ in biology has not yet been completely assessed. It has been suggested that its involvement is besides the effect of ionizing radiation in water systems,[8a] because H$^{\bullet}$ can be obtained efficiently by the reaction of electrons with dihydrogen phosphate anion ($H_2PO_4^-$) present in the biological environment.[6c] Therefore, the scenario of a peptide or protein damage due to the hydrogen atom attack needs to be considered in great detail in order to establish its contribution to the puzzling context of radical stress to biomolecules.[2] We thought that the selectivity shown by the involvement of Met residues could be conveniently evaluated in short amino acid sequences, which also allow gathering further data on the H$^{\bullet}$ reactivity. As a model peptide we considered Met-enkephalin (**1**), a neuropeptide composed by five amino acid residues discovered in 1975.[8b] We also included its leucine (Leu) analogue (instead of Met in the pentapeptide sequence) in our investigations.

[a] Dr. C. Ferreri, Dr. C. Chatgilialoglu
ISOF, Consiglio Nazionale delle Ricerche
Via P. Gobetti 101, 40129 Bologna (Italy)
Fax: (+39) 051-639-8349
E-mail: chrys@isof.cnr.it

[b] O. Mozziconacci, Prof. K. Bobrowski
Institute of Nuclear Chemistry and Technology
Dorodna 16, 03-195 Warsaw (Poland)



Tyr-Gly-Gly-Phe-Met (**1**)

Both peptides show analgesic activity by binding to δ-opiate receptors and are therefore also known as endogenous opioids. The presence of aromatic residues (Tyr or Phe) and a sulfur-containing amino acid (Met)—with the possibility of comparing analogous sequences without Met—represents an ideal case to study any selectivity of reducing species towards the sulfur-containing amino acid and/or the participation of other reactive sites in the overall molecular reactivity.

Here we report a detailed chemical radiation study of Met- and Leu-enkephalins applying reaction conditions where the H$^{\bullet}$ are the relevant reactive species, which affect the Met residues and prime a tandem peptide–lipid damage. In fact, the chemical modification of Met-enkephalin concurs with *trans* lipid formation in model membranes, providing a molecular basis for proteomic and lipidomic changes caused by radical stress.

Results and Discussion

Radiolytic production of transients:[9] Radiolysis of neutral water leads to e_{aq}^-, HO$^{\bullet}$ and H$^{\bullet}$ as shown in Equation (1). The values in parentheses represent the radiation chemical yields (G) in units of μmol J^{-1}.

$$H_2O \xrightarrow{\gamma} e_{aq}^- \ (0.27),\ HO^{\bullet}\ (0.28),\ H^{\bullet}\ (0.062) \qquad (1)$$

In order to scavenge hydrated electrons, three procedures were employed. In N_2O-saturated solutions (~0.02 M), e_{aq}^- are efficiently transformed into HO$^{\bullet}$ radicals [Eq. (2), $k_2 = 9.1\times10^9\,M^{-1}s^{-1}$]. Under these conditions, H$^{\bullet}$ and HO$^{\bullet}$ radicals accounted for 10 and 90%, respectively, of the reactive species. In O_2-free 10 mM $H_2PO_4^-$ solutions, e_{aq}^- may be converted into H$^{\bullet}$ [Eq. (3), $k_3 = 1.5\times10^7\,M^{-1}s^{-1}$], depending on the pH (pK_a values of H_3PO_4 are 2.16, 7.21 and 12.32) and the reactivity of e_{aq}^- with substrate. On the other hand, at pH 1.5, e_{aq}^- are efficiently transformed into H$^{\bullet}$ [Eq. (4), $k_4 = 2.3\times10^{10}\,M^{-1}s^{-1}$].

$$e_{aq}^- + N_2O \xrightarrow{H^+} N_2 + HO^{\bullet} \qquad (2)$$

$$e_{aq}^- + H_2PO_4^- \rightarrow H^{\bullet} + HPO_4^{2-} \qquad (3)$$

$$e_{aq}^- + H^+ \rightarrow H^{\bullet} \qquad (4)$$

In the presence of 0.2–0.5 M *t*BuOH, HO$^{\bullet}$ radicals are scavenged efficiently [Eq. (5), $k_5 = 6.0\times10^8\,M^{-1}s^{-1}$], whereas H$^{\bullet}$ react only slowly [Eq. (6), $k_6 = 1.7\times10^5\,M^{-1}s^{-1}$].

$$HO^{\bullet} + t\text{BuOH} \rightarrow (CH_3)_2C(OH)CH_2^{\bullet} + H_2O \qquad (5)$$

$$H^{\bullet} + t\text{BuOH} \rightarrow (CH_3)_2C(OH)CH_2^{\bullet} + H_2 \qquad (6)$$

The reactivity of peptides towards hydrated electrons: The pseudo first-order rate constants (k_{obs}) for the reaction of e_{aq}^- with a variety of peptides containing methionine, were determined by measuring the rate of the optical density decrease of e_{aq}^- at 720 nm ($\varepsilon = 1.9\times10^4\,M^{-1}cm^{-1}$)[10] at pH ~7. From the slope of k_{obs} versus the peptide concentration, the bimolecular rate constants $k(e_{aq}^-)$ were determined. The values are reported in Table 1 together with selected data from the literature.[11] As expected, the presence of aromatic rings and the accumulation of amide moieties increase the reactivity of peptides towards hydrated electrons.

Table 1. Rate constants for the reaction of hydrated electrons with methionine-containing peptides.

Substrate	$k(e_{aq}^-)$ [M^{-1}s^{-1}]	Ref.
Met	4.0×10^7	[9]
Met-Met	2.7×10^8	[11]
Gly-Met	4.2×10^8	[11]
Tyr-Met	$(7.7\pm0.5)\times10^8$	this work
Trp-Met	$(8.8\pm0.3)\times10^8$	this work
Met-enkephalin	$(3.8\pm0.2)\times10^9$	this work
Gly-Gly-Phe-Met	$(6.4\pm0.3)\times10^9$	this work

The reactivity of enkephalins towards hydrogen atoms: The optical absorption spectra obtained from the pulse irradiation of a Ar-purged aqueous solutions of Leu- or Met-enkephalin (0.1 mM) and *t*BuOH (0.5 M) at pH 1.5 are shown in Figures 1 and 2, respectively (open circles). Under these conditions no hydrolysis of the peptide takes place; the HO$^{\bullet}$ radicals are scavenged by *t*BuOH and e_{aq}^- are efficiently converted to H$^{\bullet}$.

From the available relative rate constants it is expected that Tyr and Phe together with Met will be the sites for the H$^{\bullet}$ attack.[4,5,12] For Met-enkephalin it can be roughly calculated that a 30–40% H$^{\bullet}$ should react with the Met residue.

In order to evaluate the relative reactivity of the two enkephalins towards H$^{\bullet}$ and the site of attack, pulse radiolysis experiments were performed in order to obtain spectra of the isolated radicals derived from Tyr, Phe and Met. Experiments were performed with a tripeptide Tyr-Gly-Gly, and two dipeptides, Phe-Leu and Gly-Met under identical conditions as for enkephalins. These peptides were selected in order to mimic an appropriate fragment of either Leu-enkephalin or Met-enkephalin and also the position with respect to the N- and/or C-terminal functions of the most vulnerable amino acids (Tyr, Phe, and Met) towards the H$^{\bullet}$ attack. The last feature seems to be important since, for example, the transient spectrum obtained from Tyr alone was not enough for the resolution of transient spectra obtained in both enkephalins, although Tyr is about two orders of magnitude more reactive than Gly. The transient spectra obtained from the peptides Tyr-Gly-Gly (dashed line in the inset of Figures 1

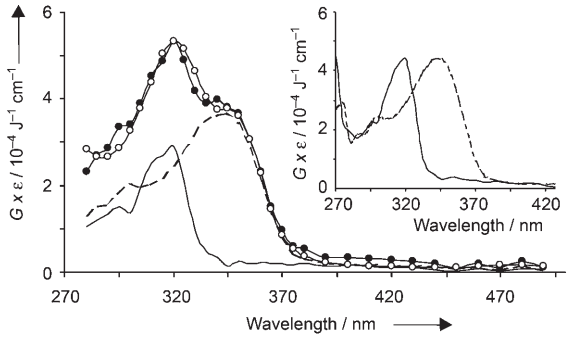


Figure 1. Absorption spectra (○) obtained from the pulse radiolysis of Ar-purged solution at pH 1.5 containing 0.1 mM Leu-enkephalin and 0.5 M *t*BuOH, taken after 10 μs after the pulse (dose = 22 Gy). The reconstruction of the spectra (●) obtained using the 62% of Tyr-Gly-Gly spectrum (——) and 38% of Phe-Leu spectrum (-----). Inset: Absorption spectra obtained under identical conditions from Tyr-Gly-Gly (——) and Phe-Leu (-----).

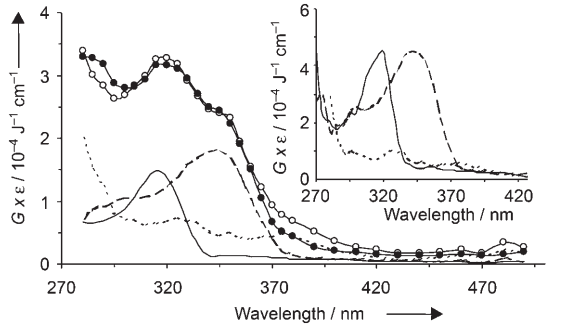


Figure 2. Absorption spectra (○) obtained from the pulse radiolysis of Ar-purged solution at pH 1.5 containing 0.1 mM Met-enkephalin and 0.5 M *t*BuOH, taken after 10 μs after the pulse (dose = 22 Gy). The reconstruction of the spectra (●) obtained using 26% of the Tyr-Gly-Gly spectrum (-----), 23% of the Phe-Leu spectrum (——) and 51% of the Gly-Met spectrum (•••••). Inset: Absorption spectra obtained under identical conditions from Tyr-Gly-Gly (——) and Phe-Leu (-----) and Gly-Met (•••••).

or 2), Phe-Leu (solid line in the inset of Figures 1 or 2) and Gly-Met (dotted line in the inset of Figure 2) were then used as possible components in the analysis of the transient spectra following reaction of H• with Leu- and Met-enkephalins. The spectra thus generated were resolved into component transients using a DECOM2003 software[13a] based on a linear regression technique.[13b] Further details of this approach have been described elsewhere.[13c]

The transient spectrum of Leu-enkephalin, which resulted from the H• attack, can be fitted properly as shown in Figure 1 (filled circles) by combining the transient spectra obtained for Tyr-Gly-Gly and Phe-Leu with the respective contribution of (62±3)% and (38±4)%. Similar spectral resolution has been performed with Met-enkephalin as shown in Figure 2 (filled circles). It was found that a good spectral resolution is achieved using the transient spectra recorded for Tyr-Gly-Gly, Phe-Leu, and Gly-Met with the following contributions: (26±1), (23±1), and (51±3)%, respectively. The important contribution of the transient species derived from Met at the lower wavelength range of the spectrum should also be noted at this stage.

When comparing the absorption spectra of the two enkephalins in Figures 1 and 2, the $G\times\varepsilon$ value measured for Met-enkephalin is found roughly equal to 62% of the value measured for Leu-enkephalin. After resolution of the Leu-enkephalin spectrum (Figure 1), the $G\times\varepsilon$ values for Tyr-Gly-Gly and Phe-Leu are 4.0×10^{-4} and $2.5\times10^{-4}\,J^{-1}cm^{-1}$, respectively. Analogously, after resolution of the spectrum for Met-enkephalin (Figure 2), the $G\times\varepsilon$ values for Tyr-Gly-Gly and Phe-Leu are 1.8×10^{-4} and $1.5\times10^{-4}\,J^{-1}cm^{-1}$, respectively. Therefore, substitution of Leu by Met reduced the amount of H• reaction with the tyrosine and phenylalanine residues by 55%.

In summary, the pulse radiolysis experiments indicated that about 50% of H• react with the methionine moiety in Met-enkephalin.

Reaction of hydrogen atom with dipeptides containing methionine in POPC-LUVET suspensions: The formation of diffusible thiyl radicals derived from the reaction of the H• with the Met moiety was monitored by using *trans* lipids as markers.[6,14] The liposomes were prepared by using 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC), in the form of large unilamellar vesicles (LUVET) of a 100 nm diameter by membrane extrusion with LiposoFast.[15,16] The appropriate dipeptide (60 μM) was added to this aqueous suspension. A small amount of *t*BuOH (0.20 M) was also added to the suspension. An alcohol concentration of less than 2% in volume is known to be compatible with the vesicle stability.[17] The oleate content of POPC was kept at 6.9 mM and the lipid/peptide ratio at 116:1, where the membrane association of peptide can be neglected.[18] The mixtures were deaerated (N_2 or Ar) or saturated with N_2O prior to γ irradiation at a dose rate of about 10.8 Gy min^{-1}. 100 μL aliquots of the suspension were withdrawn at different irradiation times for lipid isolation and derivatization to the corresponding fatty acid methyl esters.[19] GC analysis was used for the determination of *cis*/*trans* ratio (i.e., oleate/elaidate ratio).[6,15]

Initially the experiments with Gly-Met were carried out under a variety of experimental conditions. The results in Figure 3 show the percentage of *trans* isomer formed as a function of irradiation doses. In all cases the formation of the *trans* isomer increased nearly linearly with the dose exposure. In N_2O-saturated suspensions of natural water (▽) or 10 mM phosphate buffer (pH 7.0) (●) the behaviour is identical reaching a 27% of *trans* isomer after a dose of 426 Gy. Under both conditions the radiation chemical yield of H•, $G(H^{\bullet})=0.062\,\mu mol\,J^{-1}$, is the same because e_{aq}^- are scavenged by N_2O. On the other hand, 10 mM phosphate buffer suspensions deaerated by flushing N_2 or Ar at pH 7 (○) or pH 6 (□) showed an increase of *trans* isomer for a particular dose. Under these conditions, Reaction (3) plays an important role in converting e_{aq}^- to H•. At pH 6 the percentage of *trans* isomer has doubled compared with a N_2O-saturated 10 mM phosphate buffer at pH 7, which is in agreement with the nearly doubled $G(H^{\bullet})$. It is also worth noting that in deaerated phosphate buffer saline (PBS; Na_2HPO_4

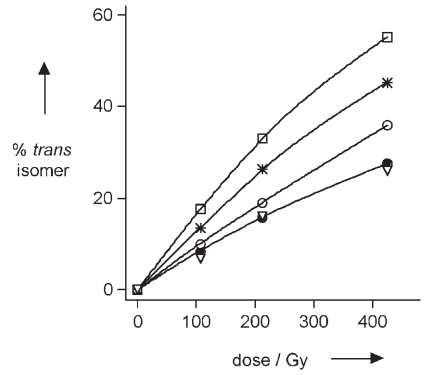


Figure 3. Dose profile of the appearance of elaidate residues (% trans isomer) from γ irradiation of POPC vesicles containing 60 μM Gly-Met and 0.2 M *t*BuOH: (▽) N_2O-saturated natural water; (●) N_2O-saturated 10 mM phosphate buffer at pH 7; (○) deaerated 10 mM phosphate buffer at pH 7; (*) deaerated phosphate-buffered saline (PBS); (□) deaerated 10 mM phosphate buffer at pH 6.

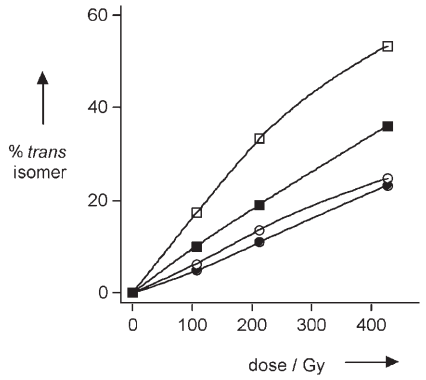
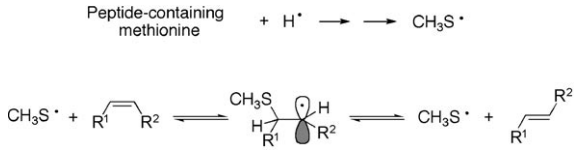


Figure 4. Dose profile of the appearance of elaidate residues (% *trans* isomer) from γ irradiation of POPC vesicles containing 60 μM peptide and 0.2 M *t*BuOH in 10 mM phosphate buffer at pH 7 and flushed with N_2. Gly-Met (■), Met-Met (□), Tyr-Met (○), Trp-Met (●).

10 mM, NaCl 0.14 M, pH 7) (*) the *trans* isomer percentage increased further compared with the analogous experiment without NaCl (●), indicating that the ionic strength of the reaction medium may also play an important role.

We suggest that the reaction of H• with the methionine residue of Gly-Met affords the diffusible thiyl radical CH_3S•.[6,7] These thiyl radicals migrate from the aqueous compartment to the lipid bilayer and transform the double bond of the PC oleate moiety, according to the catalytic mechanism shown in Scheme 1.[14,15]

The deaerated 10 mM phosphate buffer solution experiments with Gly-Met at pH 7 (■) are further compared with



Scheme 1. Methanethiyl radical-catalyzed isomerization of *cis* phospholipids.

other dipeptides in Figure 4. Two important features are observed by comparison with Met-Met (□) and with Tyr-Met (○) or Trp-Met (●). By replacing 60 μM Gly-Met with 60 μM Met-Met, the dose profile of *trans* isomer formations doubled, indicating that the concentration of thiyl radicals is now also nearly doubled. On the other hand, in the cases of Tyr-Met or Trp-Met the percentage of *trans* isomer formation diminished due to the effective competition of primary water radicals (mainly solvated electrons) with the aromatic moieties of Tyr and Trp that results in a lower $G(CH_3S•)$.

Reaction of hydrogen atom with enkephalins in POPC-LUVET suspensions: The enkephalin (60 μM) was added to the POPC-LUVET suspension prepared in a 10 mM phosphate buffer at pH 7 and in the presence of 0.2 M *t*BuOH. The mixture was deaerated (N_2-flushed) or saturated with N_2O prior to γ irradiation at a dose rate of about 10.8 Gy min^{-1}. As before, the *cis*/*trans*-lipid ratio was determined. Figure 5 shows the results with Met-enkephalin under two experimental conditions, that is, 10 mM phosphate buffer suspensions either deaerated by N_2 (▲) or N_2O-saturated (△). Comparison of the two experiments indicated that the presence of a 10 mM phosphate buffer is able to scavenge part of the electrons in the absence of N_2O. Indeed, from the above mentioned rate constants it can be calculated that 75% of e_{aq}^- have been trapped by Met-enkephalin. Therefore, a higher concentration of H• causes a higher degree of isomerization. On the other hand, through replacement of Met-enkephalin (▲) by Leu-enkephalin (◆), the isomerization is less than 1% at the highest dose (Figure 5) whereas no changes were observed (data not shown) for the replacement of Met-enkephalin by Boc-Met-enkephalin or Met-enkephalin-amide.

In Figure 5 the results for Gly-Gly-Phe-Met are also reported, which is the peptide analogous to Met-enkephalin though without the terminal tyrosine moiety. Between the two experimental conditions, that is, 10 mM phosphate buffer at pH 7 either deaerated by N_2 (●) or N_2O-saturated (○), the results are comparable with Met-enkephalin. However, the formation of the *trans* lipid with dose is slower in Met-enkephalin (▲) than in Gly-Gly-Phe-Met (●), indicating that for a certain dose a smaller concentration of the isomerising species is produced from Met-enkephalin. These findings are in excellent agreement with the pulse radiolysis results, which indicates that tyrosine in Met-enkephalin is able to scavenge about 25% of H• ions.

Conclusions

Met-enkephalin suffers from a highly selective H• attack on the Met residue. About 50% of H• attack the thioether moiety of methionine (presumably via a sulfuranyl radical)[6,20] with formation of CH_3SH and/or CH_3S• radical, that is, the diffusible isomerising radical species. In this scenario, the reaction of electrons with phosphate anions at a physiological pH generates H• efficiently and primes the occur-

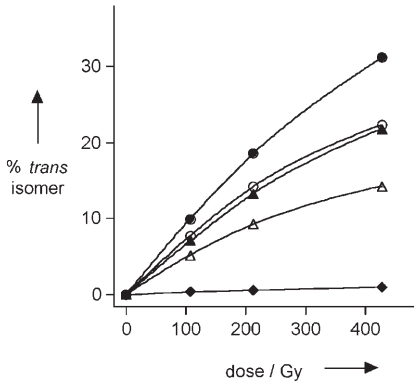


Figure 5. Dose profile of the appearance of elaidate residues (% *trans* isomer) from γ irradiation of POPC vesicles containing 60 μM peptide and 0.2 M *t*BuOH in 10 mM phosphate buffer at pH 7. Met-enkephalin in deaerated (▲) and N_2O-saturated (△) solutions; Gly-Gly-Phe-Met in deaerated (●) and N_2O-saturated (○) solutions; Leu-enkephalin in deareated (◆) solutions.

rence of tandem damage which involves the Met-containing peptides and unsaturated membrane lipids. The formation of *trans* isomers in the lipid domain acts as a biomarker of the radical damage occurring in molecules dissolved in the aqueous compartment. The coupled damage in these two compartments, as well as the functional role of chemical modifications of the lipid double bond and Met, could inspire a chemical biology approach and suggest productive correlations between studies in proteomics and lipidomics of radical stress.

Experimental Section

Materials: All peptides were purchased from Bachem, were of the purest grade available and were used as supplied. The solutions of peptides were freshly prepared immediately before each experiment.

Pulse radiolysis: Pulse radiolysis was carried out using 10 ns, 10 MeV electron pulses from an LAE 10 linear accelerator at the Institute of Nuclear Chemistry and Technology (INCT). The pulse radiolysis setup has been described elsewhere.[21] The irradiation cell was supplied with a fresh solution by continuous and controlled flow. The dose per pulse, which was determined by thiocyanate dosimetry, was 22 Gy.[22] Solutions of enkephalin (10^{-4} M) were prepared at 10 °C at pH 1.5 in the presence of 0.5 M of *t*BuOH. Water was distilled three times over permanganate. Samples were purged with argon for 30 min per 50 mL volume before the experiment and before the addition of *t*BuOH.

Continuous radiolysis: LUVET were prepared as previously described[15] from POPC (1.9 mg, 0.0025 mmol) in water (950 μL) purified with a Millipore system (Milli-Q). The peptide (0.6 mg in 50 μL of Milli-Q water) and *t*BuOH (0.2 M) were added to the LUVET suspension. The suspension was then transferred to a vial, equipped with an open-top screw cap and a Teflon-covered septum; the reaction mixture was saturated with N_2O or flushed with N_2 prior to γ irradiation. Workup and analysis of the irradiated reaction mixture were carried out as previously reported.[15]

Acknowledgements

Work supported in part by the European Community's Marie Curie Research Training Network under contract HPRN-CT-2002-000184 [SULFRAD].

[1] a) W. M. Garrison, *Chem. Rev.* **1987**, *87*, 381–398; b) C. L. Hawkins, M. J. Davies, *Biochim. Biophys. Acta* **2001**, *1504*, 196–219.
[2] B. Lipinski, *Br. J. Nutr.* **2002**, *87*, 93–94.
[3] a) B. E. Holmes, G. Navon, G. Stein, *Nature* **1967**, *213*, 1087–1091; b) R. Shapira, G. Stein, *Science* **1968**, *162*, 1489–1491; c) L. K. Mee, S. J. Adelstein, G. Stein, *Radiat. Res.* **1972**, *52*, 588–602; d) N. N. Lichtin, J. Ogdan, G. Stein, *Radiat. Res.* **1973**, *55*, 69–80; e) L. K. Mee, S. J. Adelstein, *Radiat. Res.* **1974**, *60*, 422–431.
[4] N. N. Lichtin, A. Shafferman, *Radiat. Res.* **1974**, *60*, 432–440.
[5] L. K. Mee, S. J. Adelstein, C. M. Steinhart, N. N. Lichtin, *Radiat. Res.* **1977**, *71*, 493–504.
[6] a) C. Ferreri, I. Manco, M. R. Faraone-Mennella, A. Torreggiani, M. Tamba, C. Chatgilialoglu, *ChemBioChem* **2004**, *5*, 1710–1712; b) A. Torreggiani, M. Tamba, I. Manco, M. R. Faraone-Mennella, C. Ferreri, C. Chatgilialoglu, *Biopolymers* **2006**, *81*, 39–50; c) C. Ferreri, I. Manco, M. R. Faraone-Mennella, A. Torreggiani, M. Tamba, S. Manara, C. Chatgilialoglu, *ChemBioChem* **2006**, *7*, 1738–1744.
[7] V. Kadlcik, C. Sicard-Roselli, C. Houée-Levin, M. Kodicek, C. Ferreri, C. Chatgilialoglu, *Angew. Chem.* **2006**, *118*, 2657–2660; *Angew. Chem. Int. Ed.* **2006**, *45*, 2595–2598.
[8] a) H. B. Michaels, J. W. Hunt, *Radiat. Res.* **1978**, *74*, 23–34; b) J. Hughes, T. W. Smith, H. W. Kosterlitz, L. A. Fothergill, B. A. Morgan, H. R. Morris, *Nature* **1975**, *258*, 577–579.
[9] a) G. V. Buxton, C. L. Greenstock, W. P. Helman, A. B. Ross, *J. Phys. Chem. Ref. Data* **1988**, *17*, 513–886; b) A. B. Ross, W. G. Mallard, W. P. Helman, G. V. Buxton, R. E. Huie, P. Neta, *NDRL-NIST Solution Kinetic Database—Ver. 3*, Notre Dame Radiation Laboratory, Notre Dame, IN and NIST Standard Reference Data, Gaithersburg (MD), **1998**.
[10] G. L. Hug, *Nat. Stand. Ref. Data Ser. Nat. Bur. Stand.* **1981**, *69*, 6.
[11] G. Strzelczak, K. Bobrowski, J. Holcman, *Radiat. Res.* **1998**, *150*, 688–694.
[12] W. A. Volkert, R. R. Kuntz, *J. Phys. Chem.* **1968**, *72*, 3394–3400.
[13] a) DECOM2003, P. Wisniowski, J. Mirkowski, INCT, Warsaw, Poland; b) P. R. Bevington, *Data Reduction and Error Analysis for the Physical Sciences*, McGraw-Hill, New York, **1969**; c) C. Schöneich, D. Pogocki, G. L. Hug, K. Bobrowski, *J. Am. Chem. Soc.* **2003**, *125*, 13700–13713.
[14] a) C. Chatgilialoglu, C. Ferreri, *Acc. Chem. Res.* **2005**, *38*, 441–448; b) C. Ferreri, C. Chatgilialoglu, *ChemBioChem* **2005**, *6*, 1722–1734; c) C. Chatgilialoglu, C. Ferreri in *Cis–Trans Isomerization in Biochemistry* (Ed.: C. Dugave), Wiley-VCH, Weinheim, **2006**, pp. 95–112.
[15] a) C. Chatgilialoglu, C. Ferreri, M. Ballestri, Q. G. Mulazzani, L. Landi, *J. Am. Chem. Soc.* **2000**, *122*, 4593–4601; b) C. Ferreri, C. Costantino, L. Perrotta, L. Landi, Q. G. Mulazzani, C. Chatgilialoglu, *J. Am. Chem. Soc.* **2001**, *123*, 4459–4468; c) C. Ferreri, A. Samadi, F. Sassatelli, L. Landi, C. Chatgilialoglu, *J. Am. Chem. Soc.* **2004**, *126*, 1063–1072.
[16] C. Ferreri, S. Pierotti, A. Barbieri, L. Zambonin, L. Landi, S. Rasi, P. L. Luisi, F. Barigelletti, C. Chatgilialoglu, *Photochem. Photobiol.* **2006**, *82*, 274–280.
[17] A. S. Domazou, P. L. Luisi, *J. Liposome Res.* **2002**, *12*, 205–220.
[18] M. Bokvist, F. Lindström, A. Watts, G. Gröbner, *J. Mol. Biol.* **2004**, *335*, 1039–1049.
[19] J. F. K. Kramer, V. Fellner, M. E. R. Dugan, F. D. Sauer, M. M. Mossoba, M. P. Yurawecz, *Lipids* **1997**, *32*, 1219–1228.
[20] P. Wisniowski, K. Bobrowski, I. Carmichael, G. L. Hug, *J. Am. Chem. Soc.* **2004**, *126*, 14468–14474.
[21] a) J. Mirkowski, P. Wisniowski, K. Bobrowski, *INCT Annual Report* **2000**, 31–33; b) K. Bobrowski, *Nukleonika* **2005**, *50*, S67–S76.
[22] E. Janata, R. H. Schuler, *J. Phys. Chem.* **1982**, *86*, 2078–2084.

Received: June 13, 2006
Published online: November 24, 2006

