This report is a part of the original paper [4] and was presented during the 9th International Symposium on Organic Free Radicals (Porto Vecchio, France).

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RADIATION-INDUCED MODIFICATION OF SHORT PEPTIDES MODELLING ENKEPHALIN FRAGMENTS

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Enkephalins are pentapeptides containing a tyrosine residue, two glycine residues, a phenylalanine residue, and a methionine or a leucine residue, depending on the enkephalin [1]. They are in the class of opioid peptides and play roles as neurotransmitters or neuromodulators. Moreover, they have shown immunoregulatory activity [2]. The three amino acid residues tyrosine, phenylalanine, and methionine are especially susceptible to oxidation in these peptides. Such reaction pathways are relevant to biological systems exposed to conditions of oxidative stress [3,4].

The pulse radiolysis results of enkephalins presented in our previous report are complex [5]. In order to determine the exact nature of the transient species involved in the processes following the pulse radiolysis of these pentapeptides, we oxidized model compounds having fragments that mimic different structural features of the enkephalins. Our recent studies have also focused on the interaction of the generated transients with protons.

Hydroxyl radicals (•OH), produced in the radiolysis of water, were used as one electron oxidants. The resulting transient species were examined by pulse radiolysis with optical detection. In the case of tyrosine, the hydroxyl radical reacts mainly by addition to the aromatic ring, creating the dihydroxycyclohexadienyl radical. This radical can undergo dehydration and form a tyrosyl radical, but other reaction pathways (*i.e.* disproportionation) are also possible. However, of these reactions, only dehydration can be catalyzed by protons. In our research we have concentrated on the yield and the rate of build-up of tyrosyl radicals as a function of:

- adjacent amino acid residues and/or functional groups (*i.e.* carboxyl or amino group),
- concentration of protons,
- position of selected residue in the peptide chain (*e.g.* tyrosine in N-terminal position, in C-terminal position, or in the middle of chain).

In the case of peptides containing tyrosine and methionine, the tyrosyl radical is generated in two steps, and protons accelerate only one of these steps. The first step (the faster one) is the result of an electron transfer between the radical centered on the methionine residue and tyrosine residue itself. The second step (the slower one, which is accelerated by protons) shows a pH dependence. In the peptides Tyr-Met and Met-Tyr, the specific sequence of these two residues has a distinct influence on the oxidative pathway of the dipeptides. This sequence dependence can be clearly seen in the transient spectra following pulse irradiation of these two dipeptides (Fig.1). Probably in the Met-Tyr case, radicals centered on the methionine are stabilized by three electron bonds S∴N. However, this issue needs further examination due to the overlapping of the absorption of the tyrosyl radicals with the absorption of the three electron bonded

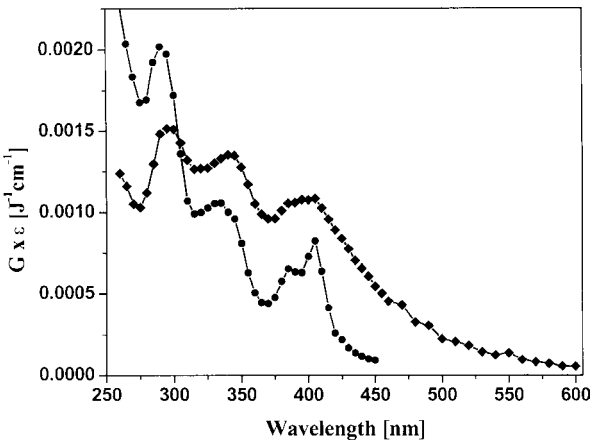

Fig.1. Transient spectra recorded 3 μs after pulse irradiation of an N_2O saturated aqueous solution containing: (◆) 0.2 mM Met-Tyr, pH 6.6; (●) 0.2 mM Tyr-Met, pH 6.1.

S∴N species. In the other peptides examined (tyrosine with others amino acid residues), no electron transfer was observed.

The rate of the dehydration reaction of the dihydroxycyclohexadienyl radical (catalyzed by protons) depends on the position of the tyrosine residue in the peptide chain. Generally, when the tyrosine residue is C-terminal, the rate of dehydration is faster than for tyrosine alone. When tyrosine is the N-terminal residue, the opposite effect can be observed. These observations can be clearly seen in the peptides Tyr-Gly and Gly-Tyr (Fig.2). The rate of tyrosyl radical formation are $k=1.8\times10^7$ dm^3 mol^{-1} s^{-1} and $k=9.4\times10^7$ dm^3 mol^{-1} s^{-1}, respectively and for tyrosine alone $k=2.9\times10^7$ dm^3 mol^{-1} s^{-1}. The hypothesis that the transient products in the reaction

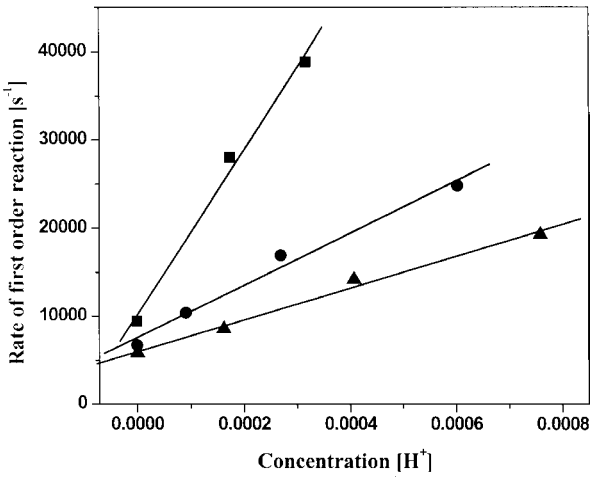

Fig.2. First order rate of tyrosyl radical formation *vs*. proton concentration for the following compounds: (■) Gly-Tyr peptide, (●) Tyr, (▲) Tyr-Gly peptide.

pathway between the dihydroxycyclohexadienyl radical and tyrosyl radical, called radical cation, is stabilized by the peptide bond or negative charge on the carboxyl group, is not confirmed by our pulse radiolysis studies with optical detection. The situation is complicated since the shape of absorption of the radical cation might be identical with the shape of tyrosyl radical [6]. This hypothesis can be confirmed most easily by the computational chemistry. Tentative computational surveys confirm this hypothesis.

In contrast, radicals centered on the aromatic ring of the phenylalanine residues are pH independent.

The following conclusions can be drawn:

- We cannot easily extrapolate the chemistry of isolated amino acids to the reactivity of their residues, even for short peptides containing highly-reactive and slowly-reactive amino acid residues like tyrosine and glycine, respectively.
- Peptide bonds and neighboring amino acid residues have some effect on the aromatic ring in the tyrosine residue.
- When the tyrosine is a C-terminal residue, the rate of reaction of dehydration is faster than when the tyrosine is N-terminal.

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ELECTRON PARAMAGNETIC RESONANCE STUDY OF RADIATION-INDUCED RADICALS IN 1,3,5-TRITHIANE AND ITS DERIVATIVES

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It is known that trithiane compounds (containing sulfur atoms) are involved in radiation-induced polymerization in the solid state. Sulfide groups increase the refractive index of the polymer due to the high polarizability of the sulfur atom, and in the polymerization product can improve its hydrophobic properties. Sulfides can also act as oxidizable compounds for the reduction of oxygen inhibition [1].

Radicals formed in gamma-irradiated 1,3,5-trithiane (TT) and its derivatives at room temperature have been studied by electron paramagnetic resonance (EPR) spectroscopy by Andrzejewska *et al*. [2].

In this paper we report results of EPR studies of radicals formed at low temperatures (77 to 293 K) in various trithiane compounds.

1,3,5-Trithiane, α-2,4,6-trimethyl-1,3,5-trithiane (α-TMT), β-2,4,6-trimethyl-1,3,5-trithiane (β-TMT), and 2,4,6-trimethyl-2,4,6-triphenyl-1,3,5-trithiane (MTFT) were prepared according to the methods described in the literature [3].

The samples were irradiated with a dose of about 4 kGy in a ^{60}Co source (Mineyola; at the Institute of Nuclear Chemistry and Technology) in liquid nitrogen. The EPR measurements were performed using a Bruker-EPS 300 spectrometer operating in the X-band (9.5 GHz) equipped with a cryostat and a variable temperature unit over the temperature range of 77-293 K.

Measurement parameters were following: microwave frequency – 100 kHz, microwave power – 1 mW, modulation amplitude – 0.2 mT.

The main component of the EPR spectrum recorded at 77 K for TT, α-TMT, β-TMT, and TMTFT was an anisotropic singlet with $g_{||}=2.011$, $g_{\perp}=2.005$ attributed to the monomeric sulfur radical cation $>S^{+\bullet}<$ (Fig.1). Similar spectral features were observed previously [4]. Warming the samples of α-TMT to 150 K resulted in the appearance of a four-line component with g=2.004 and a=1.7 mT attributed to the carbon-centered radical ($-C^{\bullet}-CH_3$) that might result from deprotonation of the mo-