REACTIONS OF OXYGEN RADICAL SPECIES WITH METHIONAL: A PULSE RADIOLYSIS STUDY *

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Summary: The reaction of hydroxyl radicals ('OH) and superoxide anions (O_2^-) with methional were investigated by pulse-radiolytic methods. The second-order rate constant for the attack of OH was determined at 8.2×10^9 M^{-1} sec^{-1}. In the case of O_2^- a slow first-order decay rate of 5.2×10^3 sec^{-1} suggests a far less efficient reaction. The transient species were identified by comparison with published results of pulse radiolysis and EPR spectroscopy of model compounds. The mechanism for the oxidation of methional by OH was found to be more complex than a simple fragmentation reaction.

Introduction: The formation of ethylene from methional (MMP) has been postulated to proceed via fragmentation of an intermediary thiyl radical cation (1). Two hypotheses presently exist for the generation of this radical species: in the reaction catalyzed by illuminated FMN or by peroxidase in the presence of several cofactors, O_2^- is considered the electrophilic agent (1,2). Conversely, for the system xanthine/xanthine oxidase it has been postulated that 'OH radicals abstract an electron from the sulfur atom (3).

This highly reactive oxydizing radical was thought to be generated in the so-called Haber-Weiss cycle (4), a concept which is increasingly gaining acceptance in biochemistry and which has been reviewed recently (5). While at the present time there are more than 24 papers suggesting or implicating a biochemical ge-

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Abbreviations: MMP - ß-methyl-mercapto-propanal; DMDS - dimethyldisulfide; EPR - electron paramagnetic resonance

neration of this radical, an important step in the cycle, the reaction

$$O_2^- + H_2O_2 \longrightarrow {}^{\cdot}OH + OH^- + O_2$$

has never actually been demonstrated, let alone evaluated. Since 1951 it has even been questioned for the catalytic degradation of H_2O_2 by ferrous ions, where it was originally proposed (6). Recently, doubts have also been raised concerning the kinetic feasibility of OH generation during the autoxidation of dialuric acid (7). Incidently, this autoxidation of dialuric acid and of 6-hydroxydopamine were the first reactions where the concomitant formation of ethylene from MMP was used as evidence for the intermediary generation of OH radicals (8). In the meanwhile, the same method was applied for the identification of OH radicals formed after illuminating chloroplasts (9).

Using a pulse radiolysis system to generate high yields of the radicals in question, we investigated the oxidation of MMP by O_2^- and OH radicals. Our results show that OH rather than O_2^- reacts with MMP; yet the mechanism is too complex to allow any further use of this method for the detection of OH radicals.

Materials and methods: MMP (Sigma) was used without further purification. Absorption spectra were determined on an UNICAM SP 800 spectrophotometer. Solutions of MMP were prepared with triply distilled and pyrolyzed water and saturated with either N_2O or O_2; some of the latter solutions also contained formate. The method of pulse radiolysis to generate high yields of the primary radicals was combined with kinetic spectroscopy to observe the absorbing transient species. The method did not allow the determination of any ethylene formed. Apparatus details have been described elsewhere (10).

Results: Solutions of MMP (1.25×10^{-5} to 6.3×10^{-3} M) were saturated with N_2O to convert all electrons to ·OH radicals - the total yield of ·OH per 100 eV ($=G_{OH}$) being 5.6; saturation with oxygen to convert the hydrated electrons (e^-_{aq}) and hydrogen atoms (·H) to O_2^- resulted in $G_{OH} = 2.8$ and $G_{O_2^-} = 3.4$. The additional presence of formate (5×10^{-2} M) converts the OH radicals to O_2^- with a combined yield of $G_{O_2^-} = 6.2$ (10). The solutions of MMP in these three systems were neutral without presence of buffers.

The transient spectra at various decay times after the pulse are depicted in Figure 1a-c. The spectra are shown without correction

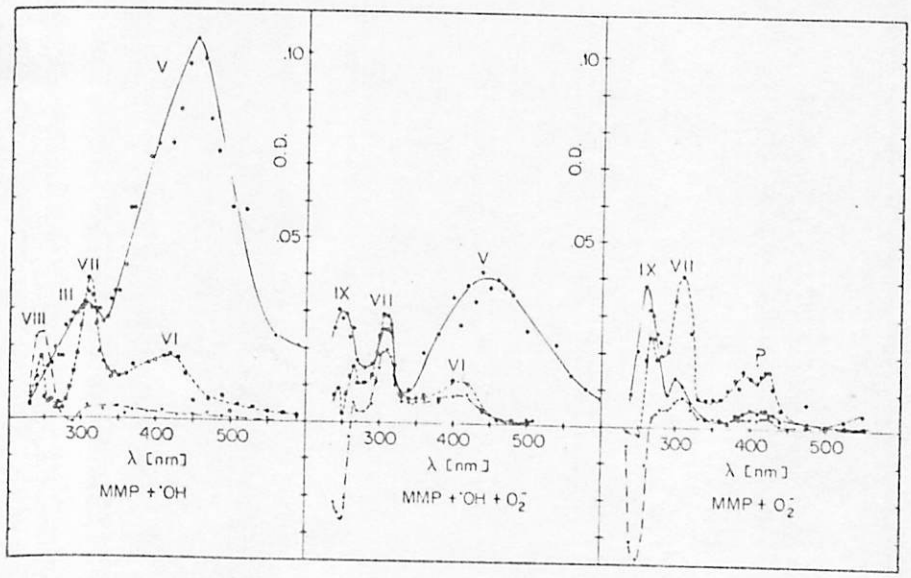


Figure 1 a-c: Transient spectra of MMP (6.3×10^{-3} M) at pH 6.8 (a) solutions saturated with N_2O; (b) solutions saturated with O_2; (c) solutions saturated with O_2, formate (5×10^{-2} M) added; solid lines (●): 10 μsec after the pulse; dashed lines (■): 100 μsec after the pulse; dotted lines (▼): 1 msec (b), resp. 10 msec (a,c) after the pulse.

(Figure 1a combines the results of two experiments after normalization for different doses per pulse; Roman numerals are the same as in the reaction scheme).

for solute depletion to demonstrate the initial presence of an unknown compound, absorbing at 250 nm. The species is formed both in aerobic and anaerobic solutions by photolysis at wavelengths below 300 nm, but it is consumed only in oxygenated solutions as evidenced from the final transmission (Fig. 1b,c). In N_2O-saturated solutions (Fig. 1a) it is obscured due to a final absorption peak at this wavelength. Though the maximum wavelength of 250 nm suggests a disulfide bridge (11), it is not dimethyldisulfide (DMDS), a co-product of ethylene during the oxidation of MMP.

Only solutions containing OH radicals (Fig. 1a,b) show the broad initial absorption peak at 450 nm. This transient, whose build-up rate could not be resolved, decays very rapidly in less than 50 μsec, leaving behind a smaller absorption peak at 410 nm. Decreasing the concentration of MMP also results in a shift from

450 to 410 nm (data not shown). The 10% H atoms, which are present in N_2O-saturated solutions, have been found to be non-reactive after complete scavenging of the OH radicals by t-butanol.

In all three systems the narrow peak at 310 nm reaches its maximum absorption at longer decay times after the pulse (100 μsec). In oxygenated solutions containing formate, i.e. where only O_2^- is present as the reactive radical, this maximum is reached only after 500 μsec, simultaneously with a double absorption peak at 390 and 420 nm.

To determine the efficiency of the relevant radicals to react with MMP, two methods had to be employed. OH, reacting very rapidly, generates the thiyl radical cation, absorbing at 410 nm at low MMP concentrations (see discussion). From the competitive inhibition of the formation of this radical by t-butanol we calculated a reaction rate of $k_{OH+MMP} = 8.2 \times 10^9\ M^{-1} sec^{-1}$, which is somewhat higher than the overall rate constant of OH with dimethylsulfide - the most simple thioether - of $5.2 \times 10^9\ M^{-1} sec^{-1}$ (12). During these experiments photolysis was prevented by use of a cut-off filter (310 nm).

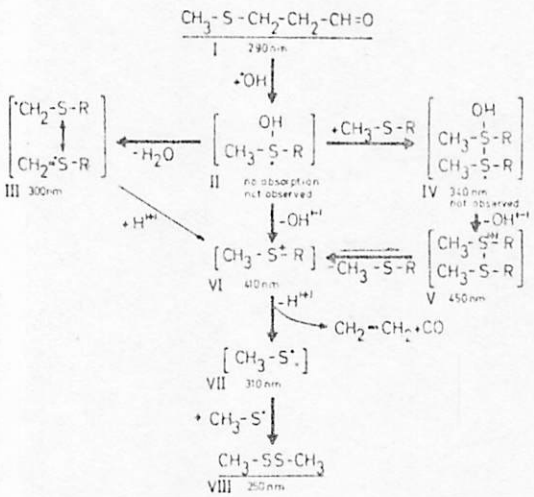
No second-order rate constant of the reaction with O_2^- could be determined as we do not know the actual concentration of either MMP or the unknown photolysis product. A first-order decay of O_2^- at 240-260 nm resulted in a rather slow consumption rate of $5.2 \times 10^3\ sec^{-1}$ (vs. $4.6 \times 10^1\ sec^{-1}$ in pure oxygenated water at pH 7), suggesting that O_2^- reacts only sluggishly with MMP.

Discussion: It is obvious from the complexity of the transient spectra under any of the employed conditions that the simple fragmentation scheme (1,3) describes only some of the reactions occurring. However, by comparing published pulse-radiolytic data of model compounds we were able to identify the majority of the transient species: oxidation of thioethers by OH radicals generally leads to the formation of dimeric thiyl radical cations $[R_2S\text{-}SR_2]^+$ (12-15) - identical with compound V of the reaction scheme. They absorb strongly at 470 nm, as compared to 450 nm in our system. The same radical has been identified by EPR spectroscopy after oxidation of thioethers by OH radicals in a modified Fenton system (16,17).

From the shift in the absorption maximum to 410 nm at lower MMP concentrations and adapting the mechanism of BONIFACIC et al. (15), we conclude that the species absorbing at 410 nm in N_2O-saturated solutions is the actual thiyl radical cation $[CH_3\text{-}S^+\text{-}R]$ (compound VI). The double absorption peaks at 390 and 420 nm in oxygenated solutions containing formate (Fig. 1c) most likely are not the same species as they show a totally different kinetic behaviour. Their identification is still in progress.

BONIFACIC et al. (15), after identifying the respective sulfoxides with a yield of 50%, do not propose any fragmentation of the radical cation VI. The reaction, however, is necessary to explain the identification of the individual fragmentation products by YANG (1). O'NEILL et al. (16) suggest the fragmentation to take place after deprotonation of the R_2S^+ monomer. The transient absorbing at 310 nm - and being only gradually formed in all of our systems - thus represents the thiyl radical VII (18, 19) rather than the carbon radical III (15) which is initially formed only in N_2O-saturated solutions (Fig. 1a).

The two remaining species both absorb at 250 nm. Compound VIII (Fig. 1a), the final absorption peak in N_2O-saturated solutions, represents DMDS, the co-product of ethylene during the OH-mediated oxidation of MMP. Compound IX (Fig. 1b,c) is identical with O_2^- as it appears only initially in oxygenated solutions.



Scheme I: Reaction mechanism for the oxidation of MMP by OH radicals

With the present data, a scheme accounting for the reaction mechanism after attack of OH radicals can be formulated:

Investigations into the nature of the still unidentified transients in oxygenated solutions respectively after photolysis (i.e. absorbing at 250 nm and at 390-420 nm) are in progress, complementing a detailed study of the O_2^--reaction both with MMP and DMDS.

We propose, however, to critically consider any further use of the assay method for the biochemical generation of OH radicals, based on the formation of ethylene from MMP. We could demonstrate that OH rather than O_2^- does react very efficiently with MMP, thus basically corroborating the hypothesis of BEAUCHAMP and FRIDOVICH (3). Yet the complexity of the oxidation mechanism with the yield of intermediary and final products being subject to changes in concentration and pH, the ease of photolytic degradation, and the likelihood that up to 50% of the OH radicals may form sulfoxides (15) - a reaction which is not without relevance for biochemical systems (20,21) - are convincing arguments against this assay.

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