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ACID-BASE PROPERTIES OF PEPTIDE PEROXYL RADICALS IN AQUEOUS SOLUTION

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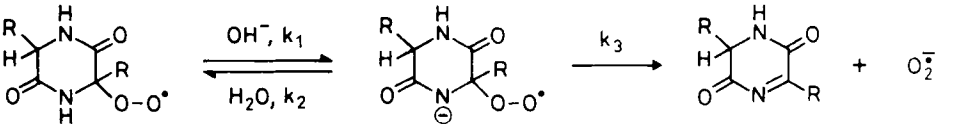
There has been considerable interest in the radiation chemistry of proteins especially in relation to protein-DNA-cross-linking as a result of free radical reactions under anoxic conditions.[1,2] In contrast very little is known about the chemistry of amino acid and protein peroxyl radicals and their possible interaction with DNA, i.e. the implication for radiation biology. We now want to focus on the acid-base properties of peptide peroxyl radicals and their subsequent reactions leading to the formation of superoxide anion radicals. This has particular relevance in view of the observation that under certain conditions the less reactive $O_2^{\bullet-}$ [3] seems to cause more damage than the OH radical itself.[4]

Recent studies on oxygen radical toxicity in *Escherichia coli* support the hypothesis that a large portion of DNA damage is mediated by a Fenton reaction that generates active forms of the hydroxyl radical from hydrogen peroxide and a Fenton-active metal which is reduced on the surface of the DNA.[5,6] Consequently in addition to H_2O_2 the damage depends on the bio-availability of both a reducing species and a transition-metal ion. Though it is not clearly established whether free hydroxyl radicals or metal-oxy complexes (ferryl radicals, "crypto-hydroxyl radicals") are involved it is generally accepted that a critical intermediate is formed leading to site-specific damage. As a source of reducing equivalents small diffusible molecules like NAD(P)H or $O_2^{\bullet-}$ should be considered.[5] For this reason peptide (protein) peroxyl radicals (for a review see Ref.[7]) might be regarded as potential precursors of transition-metal-mediated DNA damage via formation of superoxide radicals[8] (for a brief description of the chemical reactivity of superoxide radicals see Ref.[9]).

Glycine anhydride 1 and alanine anhydride 2 have been irradiated (pulse radiolysis and ^{60}Co-γ-radiolysis) in N_2O/O_2-saturated aqueous solutions. The peptide radicals formed upon $\cdot$OH attack react rapidly with O_2 ($k > 2.1 \times 10^9\,dm^{-3}\,mol^{-1}\,s^{-1}$). In basic solutions the peroxyl radicals derived from 1 and 2 deprotonate at nitrogen and the peroxyl radical anion thus formed eliminates $O_2^{\bullet-}$ ($k_3 = 1.6 \times 10^5\,s^{-1}$, and $3.7 \pm 0.9 \times 10^6\,s^{-1}$ in the case of 1 and 2, respectively (see reaction below)). The pK_a values of the peroxyl radicals have been estimated to be about 10.6 and 11.2 respectively, about three units higher than the values previously suggested.[10]



a : R = H
b : R = CH_3

A spontaneous $HO_2^{\cdot}$-elimination from the neutral peroxyl radical derived from 1 is slower than $1.2\,s^{-1}$.[11] Thus in acidic solutions the peroxyl radicals decay bimolecularly even at the low dose rates of ^{60}Co-γ-radiolysis ($2.3 \times 10^{-3}\,Gy\,s^{-1}$). At pH 4.6 the products from 1 (G values / μmol J^{-1} in parentheses) are 2,3,5-trioxopiperazine 3 (0.28), 3,4-dehydro-2,5-dioxopiperazine 4 (0.02), 2,5-dioxo-3-hydroxy-piperazine 5 (0.09) and N-glyoxylglycineamide 6 (0.17). The products 4, 5 and 6 are of the same oxidation state and in aqueous solution may possibly exist in equilibrium with one another. In alkaline solutions 4 is produced in the course of $O_2^{\cdot-}$-elimination. At a dose rate of $3 \times 10^{-2}\,Gy\,s^{-1}$ the base-induced $O_2^{\cdot-}$ elimination is even at neutral pH almost complete ($G(NF^-) = 0.52$ μmol J^{-1} in the presence of the $O_2^{\cdot-}$-scavenger tetranitromethane). In addition considerable amounts of superoxide can be formed in the course of the bimolecular decay of the peroxyl radicals via the "oxyl-route" if deprotonation at nitrogen is inhibited.[8] Pulse conductometric experiments indicate that the pK_a value of 4 (or its hydrated form 5) is $\geqslant 10.8$.

In the absence of oxygen the peptide radicals predominantly dimerise forming dehydrodimers as crosslinking products, disproportionation occurs only to a minor extent.[12]

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