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February 2, 1988

Reaction Analogues in the Radiation-Induced Deamination and Dephosphorylation of Bio-Organic Molecules II: Oxygenated Solutions

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ABSTRACT: The OH-induced deamination and dephosphorylation of simple peptides and phosphate esters in oxygenated solutions involve the formation and subsequent degradation of the peroxyl radicals $RCONHC(\dot{O}_2)R_2$ and $(P)OC(\dot{O}_2)R_2$ respectively. Reaction analogues in the degradation of peroxyl and alkoxyl radicals in these two systems are evaluated with reference to the OH-induced main-chain cleavage of protein and DNA.

In a preceeding communication[1], it was shown that the deamination of peptides by e^-_{aq} and the dephosphorylation of phosphate esters by OH in <u>oxygen-free</u> solutions are related in terms of a common elimination reaction involving free-radical intermediates of the same genre. The chemistry can be summarized by the reactions

$$H_2O \rightsquigarrow H_2O_2,\ H_2,\ OH,\ e^-_{aq},\ H^+ \tag{1}$$

$$e^-_{aq} + XCH(R)\text{-}\overset{\overset{O}{\|}}{C}\text{-}R \xrightarrow{-OH^-} XCH(R)\text{-}\overset{\overset{OH}{|}}{\dot{C}}\text{-}R \longrightarrow HX + \dot{C}H(R)\text{-}\overset{\overset{O}{\|}}{C}\text{-}R \tag{2}$$

$$OH + X'CH(R)\text{-}\underset{\underset{H}{|}}{\overset{\overset{OH}{|}}{C}}\text{-}R \xrightarrow{-H_2O} X'CH(R)\text{-}\overset{\overset{OH}{|}}{\dot{C}}\text{-}R \longrightarrow HX' + \dot{C}H(R)\text{-}\overset{\overset{O}{\|}}{C}\text{-}R \tag{3}$$

where reaction 1 represents the radiation-induced step[2,3] and X = RCONH, X′= Ⓟ0. It was also shown that these processes of HX elimination and main-chain cleavage are similarly related in the radiolysis of histone and DNA in oxygen-free systems.

The present communication extends these considerations to include the corresponding oxygenated systems. Reaction mechanisms identified in studies of main-chain cleavage in the radiolysis of peptides and proteins in oxygenated solutions are shown to have their specific analogues in the radiolytic oxidation of phosphate esters including DNA under the same experimental conditions.

Some years ago[4,5], it was shown that the radiolytic oxidation of simple peptides such as N-acetylglycine and N-acetylalanine in oxygenated solutions results in the formation of labile peptide derivatives which are readily hydrolyzed to yield (amide) ammonia and carbonyl products (keto acid plus aldehyde). The proposed mechanism[4-6] involves the preferential attack of OH at the α-carbon position

$$OH + RCONHCHR_2 \longrightarrow RCONH\dot{C}R_2 + H_2O \qquad (4)$$

followed by

$$O_2 + RCONH\dot{C}R_2 \longrightarrow RCONHC(\dot{O}_2)R_2 \qquad (5)$$

The reducing species e^-_{aq} and H formed along with OH in the radiation induced step 1 are quantitatively scavenged by O_2 to yield O_2^- and HO_2 which are related by the equilibrium $H^+ + O_2^- \rightleftarrows HO_2$. The subsequent chemistry was formulated in terms of the reactions[4,5]

$$RCONHC(\dot{O}_2)R_2 \longrightarrow RCON{=}C(R_2) + HO_2 \quad (6)$$

$$H_2O + RCON{=}CR_2 \longrightarrow RCONH(OH)R_2 \quad (6a)$$

and/or

$$HO_2 + RCONHC(\dot{O}_2)R_2 \longrightarrow RCONHC(OOH)R_2 + O_2 \quad (7)$$

$$H_2O + RCONHC(OOH)R_2 \longrightarrow RCONHC(OH)R_2 + H_2O_2 \quad (7a)$$

The dehydropeptide derivatives, $RCONHC(OH)R_2$, formed in step 6a and 7a are unstable and dissociate with cleavage of the N-C bond to yield amide and carbonyl as observed products, i.e.,

$$RCONHC(OH)R_2 \longrightarrow RCONH_2 + R_2CO \quad (8)$$

Studies of (amide) ammonia and carbonyl yields in the γ-induced oxidation of N-acyl and cyclic dipeptide derivatives of glycine and alanine in oxygenated 0.05 M solutions give $G(NH_3) \simeq G(OH) \simeq 2.8$ for each system. However, the carbonyl yields were found to be consistently low with $G(R_2CO) \leq 0.5$ in each case[5,6].

Further studies[7,8] of these model peptide systems led to the identification of a second reaction mode for removal of $RCONHC(\dot{O}_2)R_2$ radicals viz

$$2RCONHC(\dot{O}_2)R_2 \longrightarrow 2RCONHC(\dot{O})R_2 + O_2 \quad (9)$$

which occurs in parallel with reaction 6 and 7. With the N-acyl amino acids, the alkoxyl radicals formed in reaction 9 are removed in turn via

$$RCONH\text{-}\underset{\underset{R}{|}}{\overset{\overset{\dot{O}}{|}}{C}}\text{-}COOH + O_2 \longrightarrow RCONH\text{-}\underset{\underset{R}{|}}{\overset{\overset{O}{\|}}{C}} + CO_2 + HO_2 \quad (10)$$

$$\mathrm{RCONH{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle R}{|}}{C}}} + H_2O \longrightarrow RCONH_2 + RCOOH \qquad (11)$$

which results in C-C bond cleavage with the formation of compounds of higher oxidation states as <u>initial</u> products. With N-acetylalanine, the individual yields of amide, acetic acid and carbon dioxide correspond to G ~ 2.5[7]. With N-acetylglycine[8], the same chemistry occurs in essentially the same yield to give formic acid as the organic product of the oxidative cleavage reactions 9 and 10.

The importance of the self-reaction of peroxyl radicals, $2RO_2 \rightarrow 2RO+O_2$, in the radiolytic oxidation of aquo-organic systems had been formulated in an earlier report[9]. Studies of the free-radical induced autoxidation of organic substrates provided an additional source of information on the chemistry of peroxyl and alkoxyl radicals[10].

That the sequence of reactions 9-11 is not limited to the N-acyl amino acids is evidenced by studies of the radiolytic oxidation of aqueous oligopeptides and polypeptide[7,8]. With polyalanine of MW 5000, the formation of alkoxyl radicals via reaction 9 occurs more or less at random along the chain and the analogue of reaction 10 involves an adjacent peptide bond

$$O_2 + \mathrm{RCONH{-}\overset{\overset{\displaystyle O'}{|}}{\underset{\underset{\displaystyle R}{|}}{C}}{-}CONHCHR_2} \longrightarrow \mathrm{RCONH{-}\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle R}{|}}{C}}} + O{=}C{=}N{-}CHR_2 + HO_2 \qquad (12)$$

Hydrolysis of the isocyanate product of reaction 12 follows essentially instantaneously

$$H_2O + O{=}C{=}N{-}CH_2R \longrightarrow CO_2 + R_2CHNH_2 \qquad (13)$$

The use of FeIII instead of O_2 as the oxidizing scavenger in these systems results in the quantitative conversion of $RCONH\dot{C}R_2$ radicals to the dehydropeptide product[6]

$$RCONHCR_2 + FeIII \xrightarrow{H_2O} RCONHC(OH)R_2 + FeII + H^+ \qquad (14)$$

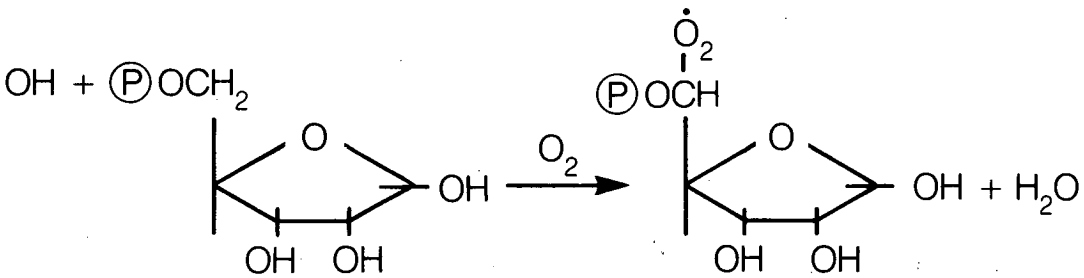
which then dissociate via reaction 8 to give (amide) ammonia and carbonyl as sole products. With N-acetylalanine: $G(NH_3) \approx G(\text{pyruvic}) \approx 3.7$. These yields are greater than $G(OH) = 2.8$ because FeII formed in reaction 14 reacts in turn with H_2O_2 formed in the radiation-induced step 1 to give an additional yield ($G \approx 0.8$) of OH radicals.

Although the reaction of OH radicals at the glycine and alanine residues occurs almost exclusively at the α C-H position at the main chain via reaction 4, with all other amino acids the side chains represent competing loci for OH attack[5,11]. Hence, the yield of main-chain cleavage in the radiolysis of proteins in oxygenated solution will be less than $G(OH) \approx 2.8$. The separation and isolation of protein fragments formed by main-chain cleavage in the radiolysis of a wide variety of proteins in oxygenated solution has recently been achieved[12-15]. These protein fragments formed with estimated G values in the range 0.3 to 1 become apparent only after reduction of S-S bonds in the irradiated protein with added RSH in aqueous sodium dodecyl sulfate.

The radiation-induced scission of the DNA strand is initiated by the formation of carbon-centered radicals at the sugar moiety through H abstraction by OH and other oxidizing radicals[16-18]. The several radicals so formed react with O_2 to give the corresponding peroxyl radicals. The chemistry of an

important sugar peroxyl radical formed at the C-5 position has been studied in some detail using ribose-5-phosphate as a DNA model[19].

In this model system, the C-5 peroxyl radical is formed via



(15)

The peroxyl radical formed via reaction 15 at the carbon position α to the phosphate group, Ⓟ$OCH(O_2)R$ has been shown to undergo a series of chemical reactions which are analogues of those identified in earlier work with the peptide radicals $RCONHC(\dot{O}_2)R_2$.

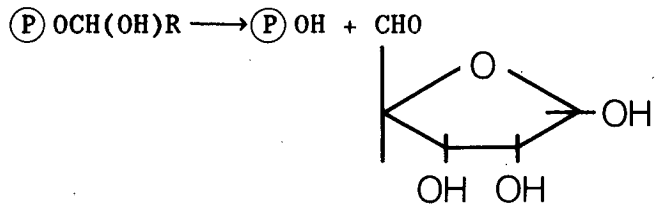
One of the paths for removal of Ⓟ$OCH(\dot{O}_2)R$ radicals formed in reaction 15 can be represented in terms of[20,21]

$$\text{Ⓟ}OCH(\dot{O}_2)R + HO_2 \longrightarrow \text{Ⓟ}OCH(OOH)R + O_2 \qquad (16)$$

followed by the hydrolysis

$$\text{Ⓟ}OCH(OOH)R + H_2O \longrightarrow \text{Ⓟ}OCH(OH)R + H_2O_2 \qquad (17)$$

The hydroxylated derivative of reaction 17 is unstable and dissociates spontaneously with cleavage of the Ⓟ O-C bond to yield free phosphate and the carbonyl product ribo-pentodialdose[19] via



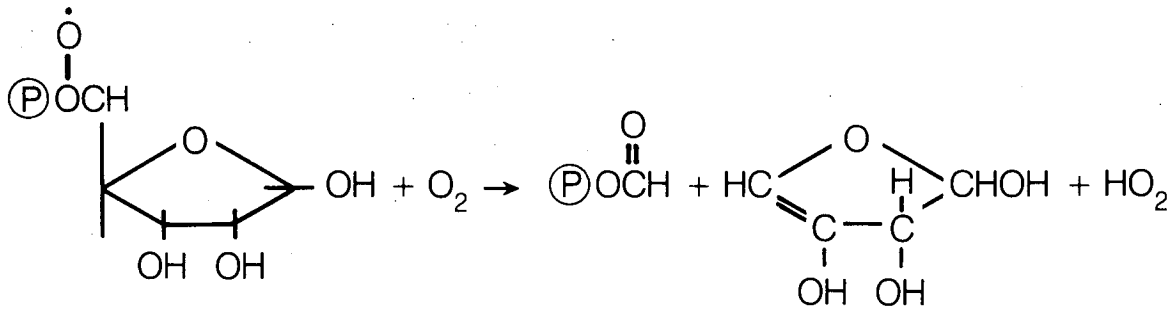
(18)

Reaction 16-18 are specific analogues of the peptide reactions 6-8 above which had been shown to yield amide and keto acid as the corresponding carbonyl product.

A second major reaction sequence proposed for removal of peroxyl radical derivaties of ribose-5-phosphate involves the self-reaction[19,20]

$$2\,Ⓟ\,OCH(\dot{O}_2)R \longrightarrow 2\,Ⓟ\,OCH(\dot{O})R + O_2 \qquad (19)$$

The alkoxyl radicals formed in step 19 react in turn with O_2 which leads to cleavage of the adjoining C-C bond with formation of formyl phosphate and the unsaturated sugar fragment erythro-tetrodialdose via[19]



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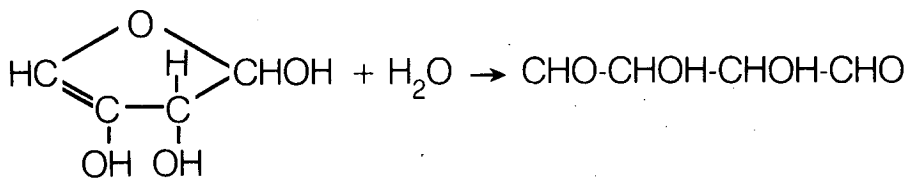

(21)

Here again there is a one to one correspondence between the general forms of reactions 19-21 and the chemistry initiated by the self-reaction of peptide $RCONHC(\dot{O}_2)R_2$ radicals outlined in reactions 9-13. About 80% of the ribose-5-phosphate radicals formed via reaction 15 are converted to erythro-tetrodialdose. This product has recently been isolated from DNA following γ-radiolysis in oxygenated solution[17,18].

As in the peptide case, the use of FeIII in place of O_2 as the oxidizing scavenger of phosphate ester radicals results in the quantitative conversion

$$Ⓟ\,O\dot{C}HR + FeIII \longrightarrow Ⓟ\,OCH(OH)R + FeII \qquad (22)$$

$$Ⓟ\,OCH(OH)R \longrightarrow Ⓟ\,OH + RCHO \qquad (23)$$

which in the case of ribose-5-phosphate radicals yields phosphate and the carbonyl product ribo-pentodialdose via reactions 22 and 23 above[22].

The development of the comparative free-radical chemistry of peptides and phosphate esters seems to be particularly appropriate at this time in view of the increasing experimental evidence that radiation damage to the chromatin nucleoprotein complex involves major chemical change in both components[23-25].

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