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RADIOLYSIS OF THE PURINE BASES
IN AQUEOUS SOLUTIONS OF FeIII

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The pyrimidine and purine moieties represent major sites of chemical degradation in the radiolysis of nucleic acids in aqueous solutions containing molecular oxygen.[1,2] Radiation chemical studies[1,3,4] of the individual pyrimidine bases indicate that OH radicals formed in the radiation-induced step

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

add to the 4,5 carbon-carbon double bond,*

$$B + OH \longrightarrow \dot{B}OH \qquad (1)$$

In oxygenated solution such addition is followed by

$$\dot{B}OH + O_2 \longrightarrow B(OH)\dot{O}_2 \qquad (2)$$

the reducing species e^-_{aq}, H, are preferentially removed to form O_2^- and HO_2 which are related by the equilibrium $HO_2 \rightleftharpoons H^+ + O_2^-$. Interactions of $B(OH)\dot{O}_2$ and HO_2 radicals then result in the formation of a multiplicity of products including 4,5 glycols, 4,5 hydroxyhydroperoxides, and other as yet unidentified products.[5] We have found recently[4] that the use of heavy-metal

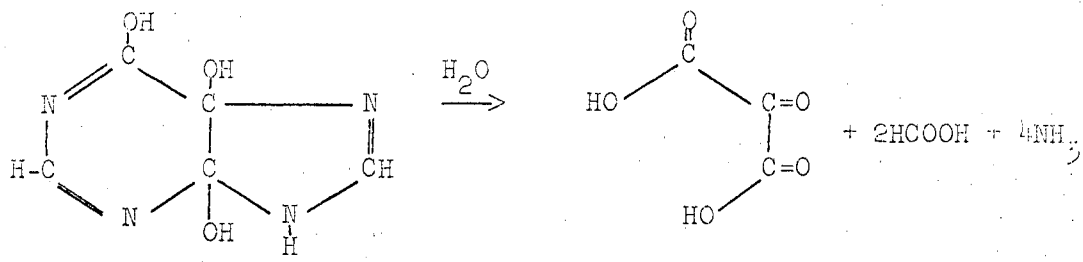
ions, CuII, FeIII, in place of O_2 as a scavenger of radical intermediates leads to a considerable simplification in the radiation chemistry of the representative pyrimidines cytosine and uracil. In the presence of FeIII the $\dot{B}OH$ radicals formed in reaction (1) are quantitatively removed to give the glycol as the sole organic product:

$$\dot{B}OH + FeIII + H_2O \longrightarrow B(OH)_2 + FeII + H^+. \qquad (3)$$

The reducing species e^-_{aq},H yield FeII. With γ-rays $G(B(OH)_2) \simeq 3 \simeq G_{OH} + G_{H_2O_2}$, where $G_{OH} = 2.4$, $G_{H_2O_2} = 0.8$ represent the 100 eV yields of OH and H_2O_2 in the radiation-induced step.

Radiolysis of the purines in oxygenated solutions leads to a complicated reaction sequence which is not well understood. Adenine, which has received the most attention is reported by Scholes and Weiss[7] to yield ammonia with $G(NH_3) = 0.5$ on radiolysis with γ-rays in aqueous solution; they suggest that such deamination arises as a consequence of OH addition to the central 4,5 carbon-carbon double bond. Miss Conlay[8] finds that the organic products from adenine include 8- hydroxyadenine and 3,4,5-triaminopyrimidine in yields corresponding to $G \simeq 0.1$. Formation of the latter products is indicative of OH attack at the 7,8 position. Such reaction is in accord with the results of Pullman and Pullman whose molecular orbital calculations[9] indicate that the 7,8 bond has the highest mobile bond order. However, since $G_{OH} \simeq 2.4$ for γ-rays it is clear that no conclusions regarding the major locus of reaction of OH with adenine can be made on the basis of the reported product yields.

We have employed FeIII in the place of O_2 as a scavenger of the intermediate radicals formed on reaction of OH radicals with the representative purine bases, adenine and hypoxanthine. Solutions containing the organic base and ferric sulfate were adjusted to the desired pH with sulfuric acid, evacuated, and irradiated with Co^{60} γ-rays at a dose rate of 6.6×10^{16} eV/gm/min for a maximum dose of 7.9×10^{18} eV/gm. Aliquots of the irradiated solutions were made 2 N in sodium hydroxide and allowed to stand 24 hours to liberate ammonia which was collected and assayed by the method of Conway.[10] Carbonyl products were determined as the 2,4-dinitrophenylhydrazones following mild hydrolysis in 2 N hydrochloric acid; identification was accomplished by filter-paper chromatography. Values of G(-B) were obtained through chromatographic isolation of the parent compound on a Dowex 50 column (1 cm × 100 cm). Representative data are summarized in Table 1. We find that the yields for destruction of adenine and hypoxanthine in the presence of FeIII are in accord with the stoichiometry $G(-B) \simeq 3 = G_{OH} + G_{H_2O_2}$ as observed earlier with the pyrimidines. The magnitude of the total ammonia yields from both adenine and hypoxanthine is consistent with OH addition to a double bond of the six membered ring via reaction akin to 1 followed by reaction of type 3. The addition product so formed is labile because of the loss of ring conjugation and on treatment with 2 N sodium hydroxide undergoes complete hydrolysis to yield the ultimate molecular components. Isolation of mesoxalic acid provides direct experimental evidence for involvement of the 4,5 double bond in the formation of a labile glycol, e.g.,



Velocity constants for reaction of OH with the purines[11] and with FeIII[12] are such that at the higher (FeIII)/(base) ratios of Table 1 an appreciable fraction of the OH radicals are removed via FeIII + OH $\longrightarrow$ FeIV + OH^-. Apparently the modes of reaction of FeIV in solutions of adenine and hypoxanthine are stoichiometrically equivalent to those of the OH radical.

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Table 1

Radiolytic Degradation of Adenine and Hypoxanthine (5mM) in Aqueous Solution Containing FeIII

	FeIII(mM)	pH	$G(NH_3)$	G(-B)	$G(CO(COOH)_2)$
hypoxanthine	50	2.0	10.4	3.0	1.7
	100	1.8	10.3		
adenine	50	2.0	11.4	2.7	0.9
	250	1.6	11.7		

Footnotes and References

*We use the notation in which this carbon-carbon double bond of both the purine and the pyrimidine nucleus is referred to as the 4,5 position.

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(5) G. Scholes, Progress in Biophysics 13, 59 (1963).

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(12) I. A. Taub and D. B. Wilson, Abstracts of Papers presented at the 151st meeting, American Chemical Society, March 1966.

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