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THE RELATIVE EFFICIENCY OF RADICALS IN RADIATION DAMAGE TO DEOXYRIBOSE

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The radiation damage to Deoxyribose was studied with a view to identify the damaging species. Our results indicate that H, e^-_{aq}, CO_2^- do not cause any appreciable damage in the absence of metal compounds and ˙OH is the sole damaging entity. Iron compounds sensitize very little O_2^- damage and CO_2^- damage could not be sensitized. In N_2-saturated solutions metal compounds increase the damage by converting e^-_{aq} into deleterious ˙OH.

KEY WORDS: O_2^-, ˙OH, radiation, deoxyribose, metal ions.

INTRODUCTION

The superoxide radical, O_2^-, is formed in most biological systems and is responsible for many deleterious effects.[1-3] However, O_2^- itself is not very reactive in aqueous solutions[4] and most of the damaging effects must be due to a more reactive species formed from it. The likely species responsible for the deleterious effects is ˙OH[5] or a higher valent state of copper or iron.[6,7,8] However, O_2^- becomes toxic mainly in the presence of metal compounds.[9-11] The ˙OH or Fe(IV) or Cu(III) are very reactive, and at least the ˙OH probably reacts with most organic and biological molecules with rates approaching diffusion controlled limits.[12] These radicals are formed in biological systems via ionizing radiation or through the Haber-Weiss or Fenton reaction.[13]

The interest in damage caused by oxygen radicals to deoxyribose is due to the importance of the reactions of these species involved in DNA damage. It is believed that the radiation damage and damage caused by many drugs in living organisms is due to the DNA damage initiated by these oxygen radicals. Hence, we have decided to study the damage to deoxyribose induced by radiation.

MATERIALS AND METHODS

All the chemicals were of analytical grade and were used without further purification. Deoxyribose and diethylenetriaminepentaacetic acid (DETAPAC) were from Sigma Chemical Co., desferrioxamine (DESFERAL) was a generous gift from Ciba-Geigy. Ferrous and ferric ammonium sulphate, EDTA, sodium formate and copper sulphate were supplied by E-Merk. All the solutions were prepared with distilled water that had been passed through a Millipore ultrapurification system. The phosphate buffer contained 0.2 M phosphate (Na_2HPO_4/NaH_2PO_4) in 0.3 M NaCl. Ferrous solution were always freshly prepared, just before use under N_2 atmosphere. Metal ions and

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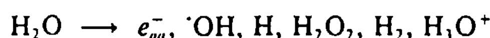
EDTA were added in the ratio of 1:1.1 for the study of the effect of the complex on damage. Thiobarbituric acid (TBA) reactivity of the damaged products of deoxyribose was tested by adding 0.5 ml of 2.8% trichloroacetic acid (TCA) and 0.5 ml of 1% TBA (w/v) in 0.5 M NaOH solution.

Results are the mean of at least three separate experiments in which the values differed by not more than 6%.

Radiation damage to deoxyribose was studied using ^{137}Cs source. The samples contained 1.1 mM deoxyribose in a phosphate buffer of pH 7.4 in the presence of 2 μM DETAPAC in order to complex metal impurities, and were irradiated in gas tight tubes with teflon seals after saturating with different gases to produce different radicals during the irradiation. TCA and TBA solutions were added to the irradiated solutions and incubated for 15 minutes at 100°C to develop malonialdehyde-like thiobarbituric acid reactive adduct.[14] The absorbance of the pink chromogen developed from the degraded products of deoxyribose was measured at 532 nm against appropriate blanks. Dosimetry was carried out with a Fricke Disometer. The yield of Fe^{3+} was measured at 302 nm with an $\varepsilon = 2197\,M^{-1}cm^{-1}$ [15] using a G-value of Fe^{3+} as 15.6. The dose rate was found to be 11 Gy/min.

RESULTS AND DISCUSSION

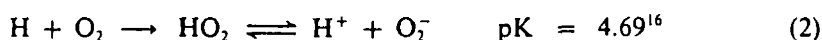
During the irradiations of aqueous solutions, the following reaction takes place:



$$H_2O \longrightarrow e^-_{aq}, {}^{\cdot}OH, H, H_2O_2, H_2, H_3O^+$$

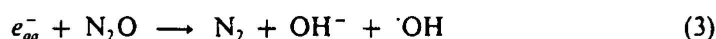
In the presence of oxygen, O_2^- is formed from the reducing radicals via

$$e^-_{aq} + O_2 \longrightarrow O_2^- \quad (1)$$



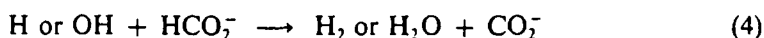
$$H + O_2 \longrightarrow HO_2 \rightleftharpoons H^+ + O_2^- \qquad pK = 4.69^{16} \quad (2)$$

When the solution is saturated with N_2O, e^-_{aq} is converted into ${}^{\cdot}OH$



$$e^-_{aq} + N_2O \longrightarrow N_2 + OH^- + {}^{\cdot}OH \quad (3)$$

and in the presence of formate, all the radicals are converted into O_2^-



$$H \text{ or } OH + HCO_2^- \longrightarrow H_2 \text{ or } H_2O + CO_2^- \quad (4)$$

$$CO_2^- + O_2 \longrightarrow CO_2 + O_2^- \quad (5)$$

TABLE I
Yields (G-values)† of Radicals formed in Irradiated dilute aqueous solutions

System	H	e^-_{aq}	$\dot{O}H$	O_2^-	CO_2^-
N_2-Saturated	0.55	2.7	2.7	-	-
Air-Saturated	-	-	2.7	3.25	-
N_2O-Saturated	0.55	-	5.4	-	-
(N_2O + O_2)-Saturated (9:1)	-	-	5.4	0.55	-
Air Saturated + Formate	-	-	-	5.95	-
N_2O-Saturated + Formate	-	-	-	-	5.95

†(G – represents the number of radicals formed per 100 eV of adsorbed energy)

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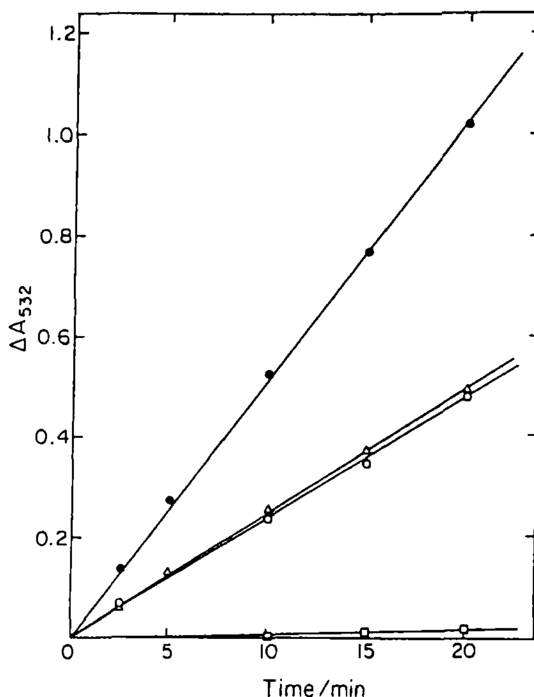

FIGURE 1 The absorbance (ΔA_{532}) of the degraded products of deoxyribose as a function of time in different systems: ○ – N_2-saturated; △ – O_2-saturated; ● – N_2O and (N_2O + O_2)-saturated; □ – N_2O-saturated + 0.1 M formate and (N_2O + O_2)-saturated + 0.1 M formate.

As a consequence of these reactions, the relative yields of the radicals depend on the solutes and saturating gases used and are given in Table I.

When buffered solutions of deoxyribose were irradiated for different intervals of time, after being saturated with various gases, the yields of the products of degradation were found to be linear with the dose (Figure 1). The yields of malonaldehyde are given in Table II. In N_2O saturated formate solutions, the only radical present is CO_2^- and in N_2O + O_2 (9:1) saturated formate solutions, O_2^- is present. The results indicate that these radicals are not reactive towards deoxyribose.

The lack of difference in the yields in N_2 and air saturated solutions shows that e_{aq}^- is not efficient in causing damage to deoxyribose. The yields in N_2O saturated

TABLE II
Yields of Malonaldehyde

Systems (aq. solution)	ΔA_{532} per minute
N_2-Saturated	0.023
Air-Saturated	0.026
N_2O-Saturated	0.054
(N_2O + O_2)-Saturated (9:1)	0.054
(N_2O + O_2) Saturated (9:1) + Formate	0.000
N_2O Saturated + Formate	0.000

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solutions clearly prove that the majority of the damage to DNA originates from ˙OH attack.

The lack of difference in damage between irradiated deoxyribose solutions saturated with N_2O and $N_2O + O_2$ (9:1) show that oxygen does not enhance ˙OH damage in agreement with DNA radiation damage *in vitro*[17] and opposed to DNA damage *in vivo*.[18]

As the metal ions and complexes such as Fe(II) and Cu(II) were known to enhance the damage to biological targets caused by O_2^- and possibly by other radicals[9-11], we have decided to study the effect of such compounds on the radiation damage to deoxyribose.

Effect of metal compounds on O_2^- and CO_2^- damage to deoxyribose

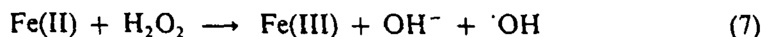
In order to determine whether metal ions and their complexes enhance O_2^- and CO_2^- damage to deoxyribose, we have studied the damage in the presence of Fe(II), Cu(II), Fe(II)EDTA and Cu(II)EDTA and the results are given in Table III. In $N_2O + O_2$ (9:1) saturated formate solutions only O_2^- is formed. It is indicated that iron complexes do enhance slightly O_2^- damage, but still in the presence of metal ions the damage due to O_2^- is negligible as compared to OH damage (Table III). In similar experiments carried out in formate solutions saturated with N_2O, no damage was observed and metal compounds had no enhancing effect on CO_2^- damage.

Radiation damage to deoxyribose in N_2 saturated solution, in the presence of metal compounds

The damage in N_2-saturated solution was shown to be mainly due to ˙OH, while e_{aq}^- and H contributes very little to deoxyribose degradation. However, when metal compounds are added, the e_{aq}^- and H reduce it as,

$$\mathrm{Fe(III)} + e_{aq}^- \text{ or H} \longrightarrow \mathrm{Fe(II)} \qquad (6)$$

and subsequently ˙OH is formed via the Fenton reaction



$$\mathrm{Fe(II)} + H_2O_2 \longrightarrow \mathrm{Fe(III)} + OH^- + {}^{\cdot}OH \qquad (7)$$

(The H_2O_2 is formed from water radiolysis).

The results in Table IV and Figure 2 show that Fe(II), Fe(III), Cu(II), Fe(III)ED-

TABLE III
Effect of metal compounds on damage to deoxyribose (ΔA_{532})

System	N₂O + O₂(9/1)saturated + 0.1 M formate					N₂O saturated
	O_2^- damage					
additives	-	Fe(II)	Fe(II)EDTA	Cu(II)	Cu(II)EDTA	˙OH damage
Irradiation time in mins.						
5	0.000	0.015	0.006	0.000	0.000	0.274
10	0.003	0.020	0.010	0.005	0.000	0.540
15	0.006	0.024	0.012	0.012	0.000	0.758
20	0.007	0.029	0.015	0.015	0.005	1.001

(The concentrations of metal ions and complexes are 20 μM).

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TABLE IV
Effect of metal ions and complexes on damage in N_2^- saturated solution (ΔA_{532})

additives	-	Fe(II)	Fe(III)EDTA	Cu(II)	Cu(II)EDTA	Fe(III)
irradiation time in mins.						
2.5	0.066	0.099	0.100	0.068	0.063	0.106
5	0.121	0.201	0.186	0.139	0.132	0.202
10	0.223	0.378	0.355	0.266	0.250	0.366
15	0.333	0.550	0.521	0.393	0.375	0.541
20	0.435	0.681	0.677	0.509	0.506	0.719

(The concentrations of all metal ions and complexes are 20 μM).

TA and Cu(II)EDTA enhance the damage, apparently through reactions (6) and (7). The damage did not depend on the concentration of deoxyribose (Table V) and also did not depend significantly on the concentration of Fe(III) complexes with EDTA. Similar results were obtained when EDTA was replaced with DESFERAL and DETAPAC.

All these results indicate that e_{aq}^- in the absence of metal ions does not contribute to damage and with the help of metal compounds, it is converted into the deleterious ·OH.

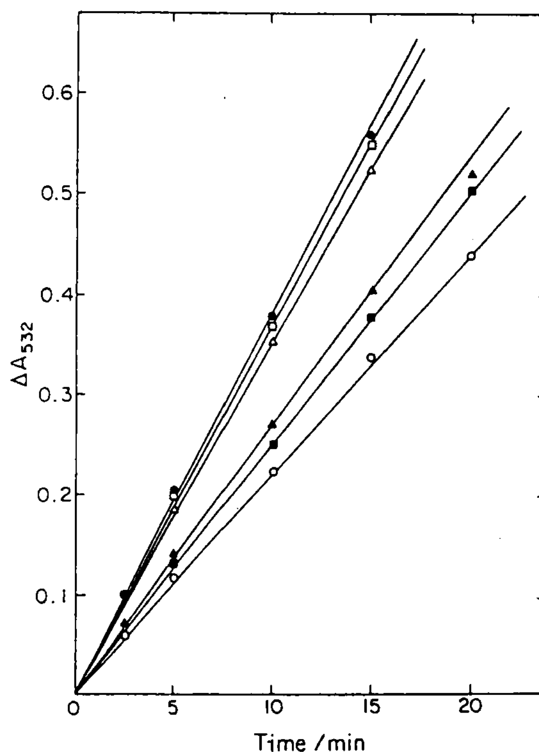

FIGURE 2 The absorbance (ΔA_{532}) of the degraded products of deoxyribose as a function of irradiation time in N_2-saturated solutions in the presence of metal compounds: ○ – without metal compounds; ● – with Fe(II); □ – with Fe(III); △ – with Fe(III)EDTA; ▲ – with Cu(II); ■ – with Cu(II)EDTA.

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TABLE V
Effect of [deoxyribose] on damage in N_2-saturated solutions

[deoxyribose] in mM	ΔA_{532} in time in mins.			
	2.5	5	10	15
0.55	0.062	0.120	0.212	0.293
1.10	0.061	0.125	0.224	0.320
2.20	0.065	0.127	0.235	0.339
5.50	0.063	0.127	0.262	0.361

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CONCLUSIONS

1) The radiation damage to deoxyribose is mainly due to $^{\cdot}OH$.

2) H, e^-_{aq}, O_2^- and CO_2^- do not cause any appreciable damage to deoxyribose.

3) Metal compounds do not enhance CO_2^- and O_2^- damage, but convert unreactive e^-_{aq} in N_2-saturated irradiated solutions into reactive $^{\cdot}OH$.

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Accepted by Prof. B. Halliwell

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