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OXYGEN ENHANCEMENT OF FREE RADICAL DAMAGE TO DNA. A COMPARISON OF RADIATION-INDUCED DEGRADATION WITH COPPER-CATALYZED DEGRADATION BY H_2O_2

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The best known concept to explain oxygen enhancement in irradiated aqueous solutions containing DNA and thiol is that of 'chemical repair' of ($^{\cdot}OH$-induced) DNA intermediates by thiols due to hydrogen donation, and competitive damage fixation in oxygenated solution due to peroxidation of DNA intermediates.[1-3] More recently it has been proposed that 'self-repair', involving electron transfer from disulphide radical anions ($RSS^{\cdot-}R$) to DNA intermediates may be of importance in irradiated oxygen-free DNA/thiol and DNA/disulphide systems;[4] oxygen enhancement can be easily explained in this case as 'self-repair' inhibition, due to scavenging of $RSS^{\cdot-}R$ and precursors (see Scheme A). Both modes of action of oxygen may possibly occur in irradiated systems. When DNA/thiol solutions are incubated with Cu^{2+}, H_2O_2 and ascorbate (at high concentration), a Fenton-type reaction of DNA-Cu(I) with H_2O_2 is thought to generate $^{\cdot}OH$ radicals immediately at the target.[5,6] The $RSS^{\cdot-}R$ precursor $RS^{\cdot}$ is scavenged by AH^- in this system,[7] and oxygen enhancement is feasible only via the 'damage fixation' mode (Scheme B).

The results shown in Table 1 demonstrate a substantial oxygen effect in γ-irradiated DNA/GSH and DNA/GSSG solutions, but no oxygen enhancement is detectable in these systems when incubated with Cu^{2+}, H_2O_2 and AH^-, despite the higher GSH or GSSG concentration — (with gamma-irradiation a higher GSH level even promotes the oxygen effect).[2] It might be suggested that H_2O_2 does not generate $^{\cdot}OH$ radicals in reaction with DNA-Cu(I). An analysis of the kinetics and yield in system B

TABLE 1
Protective effect of glutathione (GSH and GSSG) in deoxygenated (N_2) and oxygenated (O_2) DNA solutions.

A) γ-Radiolysis		B) DNA-Cu(I) + H_2O_2	
Additives (X)	PF	Additives (X)	PF
O_2	0.95	O_2	0.89
0.3 mM GSH, N_2	12.8	1.5 mM GSH, N_2	2.00
0.3 mM GSH, O_2	6.4	1.5 mM GSH, O_2	2.16
0.15 mM GSSG, N_2	10.2	1.5 mM GSSG, N_2	1.81
0.15 mM GSSG, O_2	5.1	1.5 mM GSSG, O_2	2.22

A) 25 Gy/min ^{60}Co-γ-radiolysis of 0.1 g/l DNA in 10 mM phosphate buffer; protection factors from dose(D)-effect curves: $PF = D_{37}(X)/D_{37}(O)$, with $D_{37}(O)$ = 250 Gy in oxygen-free solution.

B) Incubation at 22°C of 0.1 g/l DNA in 10 mM phosphate buffer with 10 μM $CuCl_2$, 10 mM H_2O_2 and 20 mM AH^-; protection factors from time (t) profiles of DNA degradation: $PF = t_{37}(X)/t_{37}(O)$, with $t_{37}(O)$ = 13.5 min in oxygen-free solution. After treatment A or B, 10 mM EDTA was added, and DNA degradation was tested fluorimetrically with ethidium bromide (see ref.[6]).

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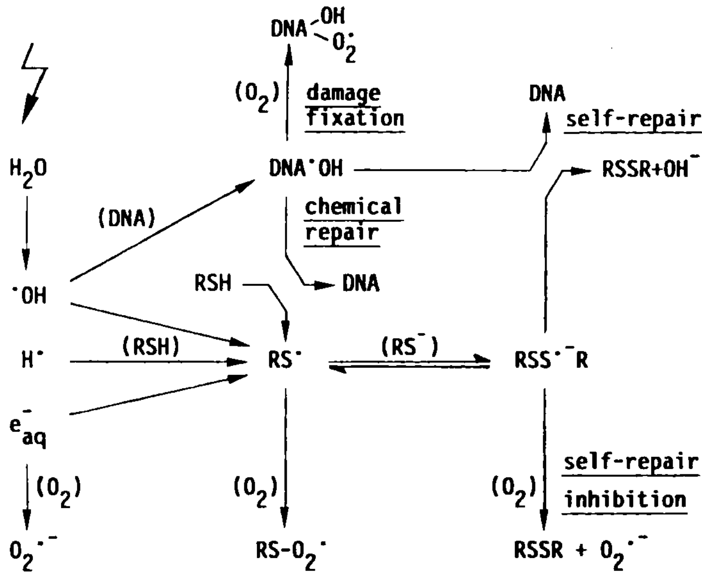

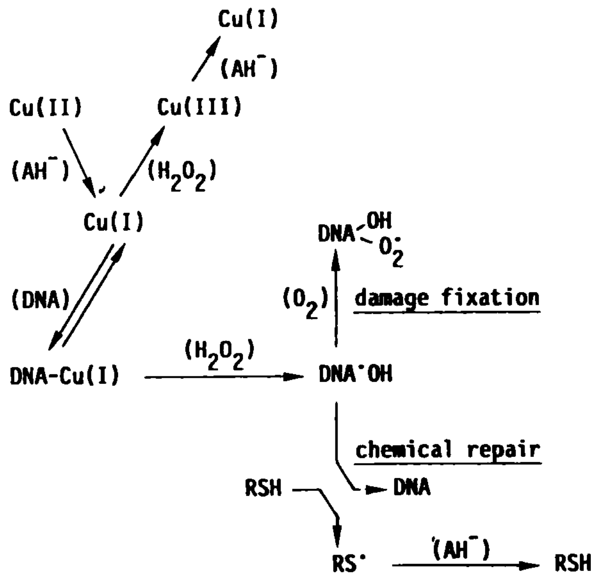

SCHEME 1

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indicated however that ·OH is the damaging entity,[6] and furthermore we have observed a characteristic fluorescence around 460 nm (under excitation at 350 nm) both in system A and B under hypoxic conditions, which can be assigned to ·OH-induced products. Therefore, to explain oxygen enhancement in γ-irradiated DNA/thiol(disulphide) solutions, the 'self-repair' concept apparently deserves further attention.

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