# Copper–DNA Complexes and their Modification by Ionising Radiation

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Addition of low concentrations of $Cu^{2+}$ ions to aqueous DNA gives units having no detectable e.s.r. spectra which, on exposure to $^{60}Co$ γ-rays at 77 K, gave a well-defined signal for $Cu^{2+}$.

Although there have been numerous studies of DNA–$Cu^{2+}$ binding,[1] hitherto it has not been postulated that there is any tendency to form $Cu^{II} \cdots Cu^{II}$ pairs. We have found that as $Cu^{2+}$ ions [$Cu(ClO_4)_2$] were added to aqueous DNA (calf thymus) at neutral pH, no e.s.r. signal was obtained for concentrations at which clear signals were detected for aqueous glasses in the absence of DNA. Further addition of $Cu^{2+}$ resulted in the growth of a well-defined $Cu^{II}$ signal (Figure 1). When a similar experiment was conducted with $Cu^{II}$ in its dipyridyl complex [$Cu(dipy)_2$], which does not interact with DNA, no such difference was seen.

It seems probable that the best binding sites for $Cu^{2+}$ ions are G–C base pairs,[2] and we tentatively suggest that at some of these sites two $Cu^{2+}$ ions can be accommodated. Once these sites are fully occupied (at a Cu : base-pair ratio of 1 : 40) further $Cu^{2+}$ ions are bound singly, presumably at isolated G–C units, and give a normal $Cu^{II}$ e.s.r. spectrum ($g_{\|}$ 2.36, $A_{\|}$ 132, $g_{\perp}$ 2.08, $A_{\perp}$ 9 G), called here type A. These suggestions are supported by the radiation chemistry of these systems. When the dilute systems were irradiated ($^{60}Co$ γ-rays at 77 K), a $Cu^{II}$ signal grew in (type B), which differs significantly from type A ($g_{\|}$ 2.31, $A_{\|}$ 153, $g_{\perp}$ 2.07, $A_{\perp}$ 14 G). Type A and type B copper spectra are shown in Figure 2b and c, respectively. The

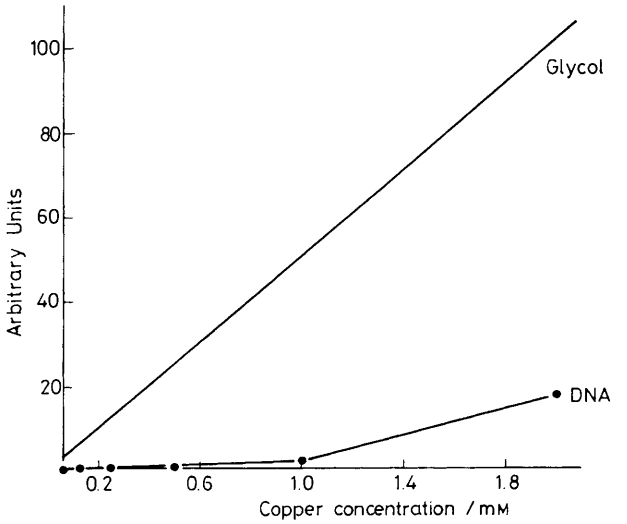


**Figure 1.** Plot showing the effect of increasing the concentration of $Cu(ClO_4)_2$ in (a) ethylene glycol glass and (b) DNA, on the double integral value of the e.s.r. signal. (DNA concentration 50 mg $ml^{-1}$.)

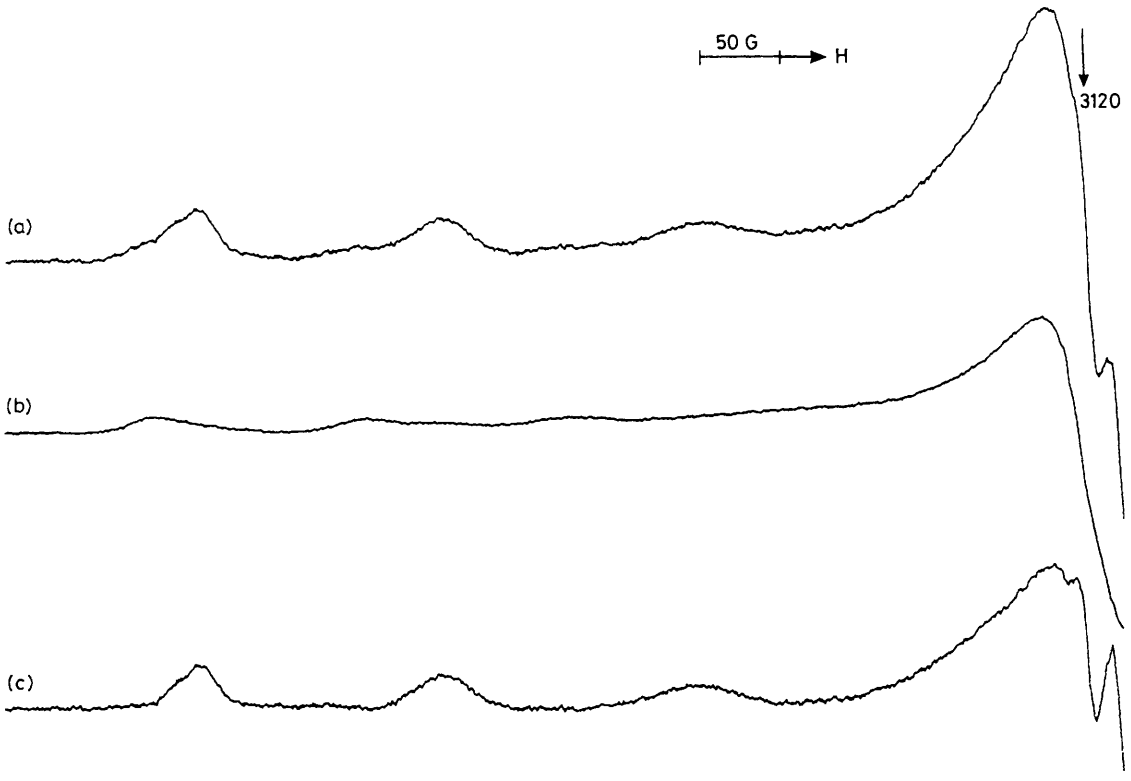


**Figure 2.** E.s.r. spectra showing the parallel region of the $Cu^{II}$ signal ([DNA] 100 mg $ml^{-1}$, [$Cu^{II}$] 2 mM); (a) shows the signal after irradiation, (b) the signal before irradiation, normalised to the amount of type A copper present in (a), and (c) the result of substracting (b) from (a), *i.e.* the amount of type B copper. The bottom of the perpendicular feature is obscured by the presence of DNA radical signals.

figures given above for the spectral parameters were found by measurement and spectral simulation.

Results from double integration of the pre- and post-irradiation spectra over the $Cu^{II}$ parallel region show that when present in low intensity, signal A decreases with irradiation whereas signal B grows in (Figure 2). On annealing to about 210 K, followed by re-cooling to 77 K for spectral recording, signal B was lost, but there was a 1 : 1 growth in A such that the intensity of A was actually greater than that before irradiation.

When samples of aqueous DNA are irradiated at low temperatures in the absence of copper only two major paramagnetic species are formed in the DNA, namely the guanine cation ($\dot{G}^+$) and the thymine anion ($\dot{T}^-$). These have been adequately characterised by e.s.r. spectroscopy.[3] For dilute copper systems, comparison of the DNA radical signals with those of a reference sample containing no copper showed that there was a clear fall in the concentration of thymine anion radicals and in the concentration of $\dot{T}H$ radicals formed therefrom, whilst little change occurred in the concentration of $\dot{G}^+$ and derived radicals. This result confirms that the major role of the $Cu^{II}$ units is to capture electrons.

We interpret the results in terms of electron capture by $Cu^{II}$ to give diamagnetic $Cu^{I}$ centres. For the $Cu^{II}$ paired units this gives a $Cu^{II} \cdots Cu^{I}$ centre, to which we assign signal B. Since hyperfine features from only one copper ion are observed there is slow electron exchange between the two copper ions, implying different co-ordination for the ions in the pair. A similar situation was found when the $Cu^{II} \cdots O_2 \cdots Cu^{II}$ unit of haemocyanin was converted to a $Cu^{II} \cdots O_2 \cdots Cu^{I}$ unit by ionising radiation.[4] The annealing results show that centre B changes readily to type A. This implies that B is a distorted form of A, and could indicate the movement of the $Cu^{I}$ centre away from its original site; or a modification of the new $Cu^{II}$ copper centre as the nature of its ligands changes.

Based on our previous studies,[5] the observed decrease in $\dot{T}^-$ and $\dot{T}H$ centres should result in a decrease in the numbers of single and double strand-breaks induced by ionising radiation, since these are the precursors of such damage. However, there was generally a significant *increase* in these breaks when copper is present during the irradiation. This is almost certainly a secondary effect. In frozen aqueous systems the radiolysis products of water, particularly $\dot{O}H$ radicals, are phase separated from the DNA and react with themselves on annealing *before* melting. In the absence of metal ions the resultant $H_2O_2$ does not lead to any additional DNA damage on thawing. When metal ions, in this case $Cu^{2+}$, are added the situation is different. The hydrogen peroxide produced on radiolysis of the ice crystallites, on thawing prior to analysis for stand breaks, react with the $Cu^{2+}$–DNA to produce the additional strand breaks detected. Experiments in which very low concentrations of aqueous hydrogen peroxide were added to unirradiated copper–doped DNA samples showed that strand breaks are extensively introduced, presumably by $\dot{H}O_2$ and $\dot{O}H$ radicals formed therefrom, thus confirming this interpretation.

Further studies will be necessary to identify the specific sites in DNA which encourage $Cu^{II} \cdots Cu^{II}$ pairing, and to see if this is a general phenomenon for different forms of DNA, and for DNA-histone complexes.

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