Interaction between glutathione and Cu(II) in the vicinity of nucleic acids

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GSH interacts with Cu(II) in the vicinity of DNA (pH $\approx$ 7) to form the DNA–Cu(I) complex, which can be quantified by characteristic absorption changes [e.g. $\Delta\epsilon_{295} = 4516 \text{ cm}^{-1} \cdot \text{M}^{-1}$ Cu(I)]. Under initial conditions of Cu(II)/GSH $\gg$ 1 and DNA(base)/Cu(II) $\gg$ 5, the stoichiometry is 1 DNA–Cu(I) per SH group (also for other thiols). Stopped-flow kinetics show that the complex is formed with half-lives of 1–30 s, depending on the environment, but independent of O₂. DNA–Cu(I) generation is much slower, less efficient, and O₂-dependent at Cu(II)/GSH < 1, or when GSH interacts with Cu(II) before the addition of DNA. Interaction of GSH with Cu(II) in the presence of DNA [at Cu(II)/GSH > 1] leads to DNA-associated transients, probably DNA–GS^{•−}–Cu(I); DNA–Cu(I) formation under these conditions is proposed to occur by ligand exchange: DNA–GS^{•−}–

Cu(I) + Cu(II) $\leftrightarrow$ DNA–Cu(I) + GS^{•−}–Cu(II). There is no evidence for generation of free thiyl radicals (GS[•]) on reaction of Cu(II) with GSH. Formation of DNA–Cu(I) is, in our opinion, a primary step involved in DNA-strand cleavage by GSH in the presence of Cu(II) [Reed and Douglas (1991) *Biochem. J.* 275, 601–608]. In this context the question of the pro-oxidative and/or antioxidative activity of GSH, when combined with copper, is discussed. GSH also generates Cu(I) complexes with other nucleic acids. An updated order of affinities of various nucleic acids for Cu(I) is presented. Cu(I) exhibits a high preference for alternating dG–dC sequences and might even be a Z-DNA inducer. The poly(C)–Cu(I) complex seems to form a base-paired structure at pH $\approx$ 7, as demonstrated by intercalation of ethidium bromide.

INTRODUCTION

Copper, a natural constituent of cell nuclei [1], has been suggested to play a key role in structural organization [2–4] and function [5,6] of chromosomes. In this context it appears important to focus attention also on the redox activity of copper in the vicinity of DNA. In a previous study [7] we have shown that Cu(II)-induced oxidation of certain compounds such as ascorbate, catechols and *o*-phenylenediamine is speeded up by orders of magnitude in the presence of DNA. This effect could be explained by the high affinity of DNA for Cu(I), which enables electron-transfer equilibria (1) between Cu(II) and electron donors D^{•−} to be driven to the right, even if thermodynamically unfeasible, by the binding equilibrium (2) removing Cu(I):

The interaction of Cu(I) with DNA, with preference for alternating dG–dC sequences [8], leads to changes in u.v.-absorption and c.d. spectra [9,10], which resemble those accompanying a conformational change from right-handed B-DNA to left-handed Z-DNA. The spectroscopic properties of the DNA–Cu(I) complex [9,10] were indeed known years before the Z-DNA structure was discovered [11,12], and it is probably not by chance that fixation of Cu(I) and B $\rightarrow$ Z DNA transitions are both favoured in alternating dG–dC sequences. Reduction of Cu(II) in the vicinity of DNA might actually serve as a functional trigger [9] to change the DNA structure, and it has already been pointed out [7] that GSH is a possible endogenous reductant.

Another aspect is that DNA in combination with copper could promote the bioreductive activation of genotoxic compounds, as depicted by the coupled reactions (1) and (2), to produce free radical metabolites (D[•]) and their progeny in the most vulnerable cell compartment, the nucleus. Furthermore, with formation of DNA–Cu(I) the DNA becomes predisposed to oxidative damage

by H₂O₂, via Fenton-type reactions that generate strongly oxidizing radicals such as [•]OH or Cu[•]O₂H [13] *in situ*, i.e. near the site of Cu(I) fixation within the DNA [8,14–17]:

↓
Damage

Copper ions are much more efficient at promoting DNA damage by H₂O₂ than iron ions [15,17–19], and this is probably due to the high affinity of DNA for Cu(I). Also O₂-dependent DNA-strand cleavage by thiols is known to be activated specifically by copper ions [20–22].

In the present paper we show that, under certain conditions, Cu(II) interacts with GSH in the vicinity of DNA to generate the DNA–Cu(I) complex. Formation of DNA–Cu(I) is in our opinion a primary step involved in DNA-strand cleavage by GSH in the presence of Cu(II) [21]. In this context we also present an updated order of affinities of various nucleic acids for Cu(I).

MATERIALS AND METHODS

Chemicals

The following commercial products were used as received: DNA (salmon testes), DL-cysteine, cysteamine (2-mercaptoethylamine), dithiothreitol (DTT), dopamine, D(–)-penicillamine, poly(dG–dC)·poly(dG–dC) with alternating dG–dC sequence, poly(A), poly(C), poly(I) and poly(U) from Sigma Chemie; L-ascorbic acid, CuCl₂·2H₂O, SDS, ethidium bromide (ETB), GSH, GSSG, Hepes, NADH, superoxide dismutase (SOD) from bovine erythrocytes and Tris from Serva Feinbiochemica. Heat-denatured DNA was obtained by 10 min boiling of DNA stock solutions and subsequent shock cooling in ice. Other chemicals

Solution A	Solution B
0.2 g/l DNA	100 μ M GSH (pH $\approx$ 7)
10 mM Tris (pH 7.4)	$\pm$ Additive
200 μ M CuCl ₂	

Solutions **A** and **B** were air-saturated (unless otherwise stated) and mixed 1:1 (v/v), with the rapid-mix/stopped-flow system thermostatically controlled at 20 °C.

Scheme 1 Standard conditions for stopped-flow experiments

such as salts and gases (N₂, N₂O and O₂) were of the highest purity available.

Generation and detection of Cu(I)–nucleic acid complexes

Cu(I)–nucleic acid complexes can be generated by reducing Cu(II) with, for instance, ascorbate [9,10,15] or catechols [7] in the presence of the nucleic acid. The Cu(II) $\rightarrow$ Cu(I) transition in combination with reaction (2) leads to a remarkable absorbance change: the absorbance difference spectrum on DNA–Cu(I) formation at Cu(I)/base ratios of $r < 0.2$ exhibits a prominent peak at 295 nm with $\Delta\epsilon = 4516 \text{ cm}^{-1} \cdot \text{M}^{-1} [\text{Cu(I)}]$, an isosbestic point around 270 nm, and a minimum (negative $\Delta\epsilon$) around 255 nm; the above value of $\Delta\epsilon_{295}$ was derived from a plot of ΔA versus [Cu(I)] using γ -radiolysis to generate DNA–Cu(I) (see below and [8]). The characteristic absorbance change enables a convenient (quantitative) identification of the DNA–Cu(I) complex, if the reductant does not mask this absorption. Absorbance changes induced by Cu(I) complexation with other nucleic acids are remarkably different from those for DNA [8,23].

Although the present study deals with DNA–Cu(I) generation by thiols, we have also applied radiolysis of solutions containing DNA, Cu(II), formate and O₂ to generate the complex [8,23,24]:

The advantage of this method is that no absorbance change occurs above 210 nm with the conversion of HCOO[−] into CO₂, and that the concentration of Cu(I) is simply determined by the radiation dose; with the primary free-radical yields (in $\mu\text{M}/\text{Gy}$ [25]), indicated in parentheses in eqn. (4), the yield of Cu(I) is 0.62 $\mu\text{M}/\text{Gy}$. Pulse radiolysis of this system has actually enabled us to estimate the rate of reaction (2): $k_2 \approx 4 \times 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$ [8]. In the present investigation we have used a ⁶⁰Co γ -source (Gamma-cell 220, Atomic Energy of Canada Ltd.) operating at 13 Gy/min.

Stopped-flow experiments

Stopped-flow measurements were performed with an SFA-12 'Rapid Kinetics Stopped-Flow-Accessory' (Hi-Tech Scientific Ltd.), coupled with a u.v.–visible spectrophotometer (Shimadzu Corporation). The flow-through mixing cell was of 1 cm optical path. Starting from the 'standard conditions' given in Scheme 1, we have varied the concentrations of the components, the buffers, the temperature and O₂ concentration, and tested other thiols

and nucleic acids. Solutions were freshly prepared for each experiment, and anaerobic conditions were obtained (if desired) by flushing the components gently with high-purity N₂, before filling the (gas-tight) syringes of the stopped-flow system. We have noted that the DNA becomes progressively denatured when solution A remained longer than 10 min in the Teflon capillaries of the rapid-mix flow system. The system was therefore loaded with fresh solutions for each 'shot'.

Figure 1 Cu(I)-induced absorbance changes (spectrum a) for native DNA, and (spectrum b) on mixing DNA–Cu(I) with poly(dG-dC)·poly(dG-dC)

The results were obtained by γ -irradiation (75 Gy) of an air-saturated solution containing 0.05 g/l DNA, 0.1 mM CuCl₂ and 2 mM sodium formate in 10 mM phosphate buffer (pH 6.8). The irradiated DNA solution was mixed 1:1 (v/v) with water (spectrum a) or with a solution containing 0.05 g/l poly(dG-dC)·poly(dG-dC) allowing 8 min for the transfer of Cu(I) from DNA to poly(dG-dC)·poly(dG-dC) (spectrum b). Spectrum b was also obtained by radiolytic generation of Cu(I) directly in the presence of poly(dG-dC)·poly(dG-dC). All spectra were recorded against the corresponding unirradiated system, using a 1 cm cell. Inserted are time profiles of absorbance changes at 245 and 295 nm caused by the Cu(I) transfer.

Increasing affinity for Cu(I)	
	↓
	↓
Poly(dT)	Poly(U)
Poly(dG)·poly(dC)	
Poly(dA-dT)·poly(dA-dT)	Poly(A)
Poly(dA-dC)·poly(dG-dT)	Poly(G)
DNA (salmon testes)	Poly(C)
Poly(dG-dC)·poly(dG-dC)	Poly(I)

Figure 2 Order of increasing affinity of nucleic acids for Cu(I)

The affinity of nucleic acids for Cu(I) is expressed by the stability of the Cu(I) complex, which ranges from minutes in the case of poly(U) to days in the case of poly(I), and by the observation of Cu(I) transfer from nucleic acids of low to those of higher affinity [8]. Cu(I) transfer between the following nucleic acids has been detected by inspecting the concomitant absorbance changes: poly(dT) $\rightarrow$ poly(G), poly(A) $\rightarrow$ poly(G), poly(A) $\rightarrow$ poly(C), poly(dA-dT)·poly(dA-dT) $\rightarrow$ poly(dG-dC)·poly(dG-dC) [8], poly(dA-dC)·poly(dG-dT) $\rightarrow$ poly(C), poly(C) $\rightarrow$ DNA [23], poly(C) $\rightarrow$ poly(I), DNA $\rightarrow$ poly(dG-dC)·poly(dG-dC) (Figure 1) and DNA $\rightarrow$ poly(I) [23].

Figure 3 Stopped-flow time profiles and spectrum showing absorbance changes caused by formation of DNA–Cu(I) by the interaction of GSH with Cu(II) in the presence of DNA in air-saturated solution

Time profiles (a) at 250 nm for native and heat-denatured DNA and the difference spectrum (b) were obtained for the standard conditions (Scheme 1); the spectrum was recorded against a blank without GSH in solution B, 1 or 60 min after mixing. (c and d) Time profiles for this system at 295 nm at various [GSH] and various temperatures (100 μ M GSH) respectively.

RESULTS

Affinity of nucleic acids for Cu(I)

The strong interaction between Cu(I) and DNA [reaction (2)] has been implied to activate certain redox reactions of Cu(II) in the vicinity of DNA [7]. Only a few attempts have been made, however, to quantify the strength of interaction of Cu(I) with various nucleic acids. Here we briefly summarize our current knowledge on this topic.

Cu(I)–nucleic acid complexes are stable in terms of minutes to days [at Cu(I)/base ratios $r < 0.2$] and are strongly dependent on base composition [8,23] and to some extent on O_2 concentration. Only for the complex of DNA with Cu(I) has an estimate of the stability constant been obtained, from rate constants of the forward and back reaction (2): $K_2 = k_2/k_{-2} \approx 2 \times 10^9 \text{ M}^{-1}$ (base) [8]. Fixation of Cu(I) by DNA prevents disproportionation of Cu(I), and efficiently inhibits the catalytic activity of copper in combination with O_2 [7]. Absorbance changes induced by Cu(I) addition to nucleic acids are also characteristically dependent on base composition. This has enabled us to demonstrate Cu(I) transfer between various nucleic acids [8,23].

Absorbance changes caused by the transfer of Cu(I) from DNA to poly(dG-dC)·poly(dG-dC) are shown in Figure 1, together with time profiles for this transition. The differences between the ΔA spectra of the complexes of Cu(I) with poly(dG-dC)·poly(dG-dC) (spectrum b), and with DNA (spectrum a), were only seen at high Cu(I)/base ratios ($r \approx 1/3$), as applied in Figure 1. At lower ratios ($r \approx 1/20$) the ΔA spectra for those two

polymers were almost indistinguishable. These results corroborate our previous suggestion [8] that Cu(I) fixation in DNA occurs preferentially in alternating dG-dC sequences.

An order of increasing affinity of deoxyribo- and ribo-nucleic acids for Cu(I), based on previous [8,23] and new results, is shown in Figure 2. In an experiment as in Figure 1, but with DNA replaced by poly(I), we have found evidence for incomplete Cu(I) transfer between poly(dG-dC)·poly(dG-dC) and poly(I). These two polymers are now considered to have the highest affinity for Cu(I).

Formation of the DNA–Cu(I) complex by GSH

DNA cleavage by GSH in the presence of Cu(II) has been observed, particularly at $[Cu(II)]_0 > [GSH]_0$ [20–22]. Under these conditions, taken as standard (Scheme 1), fast and efficient formation of DNA–Cu(I) by GSH can be detected, as shown in Figure 3. The difference spectrum (Figure 3b) developed within about 1 min of mixing, was unaltered after 60 min and exhibits the characteristic features of DNA–Cu(I) (see Figure 1 and the Materials and methods section); oxidation of GSH to GSSG contributes only weak absorbance changes below 280 nm. From the absorbance peak at 295 nm, we can estimate (with $\Delta\epsilon_{295} = 4516 \text{ M}^{-1} \cdot \text{cm}^{-1}$) that about 95% of GSH has led to formation of DNA–Cu(I). The stopped-flow time profiles in Figures 3(a) and 3(c) are monoexponential with half-lives of less than 10 s. A striking feature is that the formation of DNA–Cu(I) is faster (factor ≈ 3) for denatured than for native DNA. First-order

Table 1 First-order rates of formation of DNA–Cu(I)

Results are calculated from 295 and 250 nm time profiles of stopped-flow experiments as in Figure 3 (see Scheme 1). Values given are concentrations after mixing; [DNA] = 0.1 g/l. T, Tris (pH 7.4); H, Hepes (pH 6.8); P, phosphate (pH 6.8).

No.	CuCl ₂ concn. (μM)	GSH concn. (μM)	Buffer	Temperature (°C)	Additive	k (s ⁻¹)
1	100	50	5 mM T	20		0.09 ± 0.01
2	100	30	5 mM T	20		0.16
3	100	15	5 mM T	20		0.30
4	100	50	5 mM T	20		0.27*
5	60	30	5 mM T	20		0.08
2	100	30	5 mM T	20		0.16
6	180	30	5 mM T	20		0.29
7	100	50	5 mM T	15		0.06
8	100	50	5 mM T	30		0.17
9	100	50	5 mM T	40		0.58
10	100	50	5 mM T	20	100 μM ZnSO ₄	0.09
11	100	50	5 mM T	20	0.1 M NaCl	0.05
12	100	50	5 mM T	20	50 μM GSSG	0.02
13	50	25	5 mM H	20		0.11†
14	50	25	5 mM H	20	100 μM ZnSO ₄	0.21
15	50	25	5 mM H	20	0.05 M NaCl	0.24
16	100	50	10 mM P	20	O ₂ or N ₂	0.09
17	100	50	94 mM P	20		0.02

* Heat-denatured DNA.

† The rate was not affected by solubilizers such as Tritons X-100 (1%), SDS (10 mM) or ethanol (0.1 M).

Figure 4 Stopped-flow time profile (a) and spectra (b) showing formation of DNA–Cu(I) when DNA is added as the last component to solutions containing Cu(II) and GSH (air-saturated)

Concentrations were as in Scheme 1, but with CuCl₂ added to solution B, 3 min before mixing with the DNA solution A (without CuCl₂). Time profiles at 250 nm show the slow formation of DNA–Cu(I), in comparison with the fast formation (dotted curve) under standard conditions as in Figure 3(a). Spectra were recorded against blanks without GSH in solution B at 4 and 16 min after mixing respectively; the dotted spectrum in (b) was obtained without DNA in solution A.

rates of DNA–Cu(I) formation, obtained for a variety of conditions, but with $[\text{Cu(II)}]_0 > [\text{GSH}]_0$, are collected in Table 1. The rates increase proportionally with $[\text{Cu(II)}]_0$ but inversely with $[\text{GSH}]_0$, and there is a quite dramatic increase with temperature. The choice of buffers seems not to be very critical in the absence of additives; however, the effect of additive (see details below) depends on buffering.

It should be noted that after stopped-flow the rate of build-up of A_{295} (Figure 3c) correlates well with the rate of loss of A_{250} (Figure 3a). It may appear surprising that in the time profiles the gain in A_{295} is less than the loss in A_{250} , whereas the opposite is seen in the final difference spectrum (Figure 3b). The reason is

obviously that transients absorbing around 250 nm are formed during mixing, and that these species disappear after stopped-flow simultaneously with the appearance of DNA–Cu(I), both contributing a negative absorbance change at 250 nm. Some further details about DNA–Cu(I) precursors are presented below.

Figure 4(a) shows that generation of DNA–Cu(I), as detected by the change in A_{250} , is slower by a factor of more than 10 when Cu(II) is mixed with GSH shortly (3 min) before adding DNA, (other conditions as in Scheme 1). The final yield of DNA–Cu(I), as seen from the spectrum in Figure 4(b), is, however, comparable with that in Figure 3(b). In the absence of DNA, the interaction of GSH with Cu(II) generates products with broad absorption

Figure 5 Stoichiometry of DNA-Cu(I) formation by interaction of Cu(II) with various thiols in comparison with DNA-Cu(I) formation by ascorbate

The results refer to DNA-Cu(I) generation in air-saturated solution containing (final concentrations): 0.1 g/l DNA, 150 μ M CuCl₂, 10 mM phosphate (pH 6.8) and thiol or AH⁻. The time required to complete DNA-Cu(I) formation is indicated below. $\square$, AH⁻ (control), 1.5 min; +, DTT, 40 min; $\circ$, cysteamine, 2 min; $\times$, cysteine, 2 min; $\bullet$, GSH, 2 min; $\triangle$, penicillamine, 2 min.

bands, as shown by the dotted spectrum in Figure 4(b); these products disappear within 10 min in oxygenated solutions, but they are fairly stable under anaerobic conditions. The formation of DNA-Cu(I) became slower and less efficient the longer GSH had been in contact with Cu(II) under aerobic conditions, before addition of DNA. These results indicate that the interaction between GSH and Cu(II) proceeds O₂-dependently through various stages, and that DNA accepts Cu(I) most readily at an initial stage, i.e. when GSH interacts with Cu(II) in the vicinity of DNA, as in Figure 3.

Stoichiometry of DNA-Cu(I) formation by various thiols

Figure 5 presents a plot of the final DNA-Cu(I) absorbance (295 nm) generated by interaction of Cu(II) with various thiols in the presence of DNA; it was confirmed in each case that the reaction produces the characteristic absorbance change, as in Figure 3(b). Reduction of Cu(II) by thiols is compared with reduction by ascorbate, for which a stoichiometry of 2 Cu(I)/AH⁻ has been established in combination with DNA as Cu(I) acceptor [9]; DNA begins to saturate at 1 Cu(I) per 5 DNA bases (i.e. > 25 μ M AH⁻ in Figure 5). For all thiols tested, the stoichiometry {at [Cu(II)]₀ $\gg$ [GSH]₀} was close to 1 DNA-Cu(I) per SH group (as indicated above), and the complex was fully developed within less than 2 min, except in the case of DTT where the reaction required more than 30 min.

It must be pointed out that the stoichiometry of 1 DNA-Cu(I) per SH group is only valid if [Cu(II)]₀ $\gg$ [-SH]₀. For [Cu(II)]₀ $\leq$ [GSH]₀ the interaction between GSH and Cu(II) in the presence of DNA becomes very complicated. At [GSH]₀/[Cu(II)]₀ = 2, for instance, long-lived products with broad absorption bands were formed immediately after mixing (Figure 6b, 4 min spectrum); similar absorption bands were also seen in the absence of DNA (see also Figure 4b). These products are obviously precursors of DNA-Cu(I), as they disappear in a time scale of 1 h, concomitantly with the formation of the characteristic DNA-Cu(I)

absorption (Figure 6b). The absorbance change after 60 min indicates, however, that only about 50% of the Cu(II) was converted into DNA-Cu(I). Time profiles (Figure 6a) are complex because of the overlap of primary and secondary product absorptions. At [GSH]₀/[Cu(II)]₀ > 2 the rate and efficiency of DNA-Cu(I) formation decreased even further and time profiles (as in Figure 6a) were strongly dependent on O₂.

Effects of environment on interactions between GSH and Cu(II)

Using conditions as in Scheme 1, the rates of DNA-Cu(I) formation in oxygenated and deoxygenated solutions were identical (Table 1, no. 16). DNA-Cu(I) is less stable, however, under aerobic conditions [8], and we have noted that the complex is destabilized even more in the presence of both O₂ and SOD. Similar results obtained with poly(C) are presented and discussed below.

The increase in the rate of GSH-induced DNA-Cu(I) formation with temperature (Table 1, nos. 1, 7–9) could not be fitted to a simple Arrhenius plot. The remarkable temperature effect might actually be due to activation of several processes; also partial unfolding of the DNA should speed up DNA-Cu(I) formation (see Table 1, no. 4).

It is known that Zn(II) interferes with redox interactions of thiols with iron [26,27]. The interaction between GSH and Cu(II) in the vicinity of DNA seems, however, not to be very sensitive to Zn(II) under standard conditions (Table 1, no. 10). There was some retardation of DNA-Cu(I) formation at high [NaCl], and a drastic inhibition caused by GSSG (Table 1, nos. 11 and 12). However, the final yield of DNA-Cu(I) was only slightly reduced (10%) in the presence of GSSG. When the buffer was changed from Tris to Hepes, the formation of DNA-Cu(I) was speeded up in the presence of both ZnSO₄ and NaCl (Table 1, nos. 13–15). Hepes and other piperazine buffers are known to promote Fenton-type reactions between Cu(II) and H₂O₂ [24], but it is not clear whether this is the reason for the above effects. Generally it can be expected that additives, buffers included, can act as competitive Cu(II) and/or Cu(I) ligands to modify the reaction scenario in DNA/GSH/Cu(II) systems. High phosphate concentrations for instance slow down DNA-Cu(I) formation by GSH (Table 1, no. 17), probably because of complexation of Cu(II).

Evidence against formation of free thiyl radicals (GS[•]) by Cu(II)

In the literature we commonly encounter the proposal that redox-active transition metals such as Cu(II) and Fe(III) interact with thiols to form free thiyl radicals, which subsequently dimerize to the disulphide [28–32], e.g.:

In a recent study on GSH-mediated electron transfer [33] we have found evidence that Cu(II)-induced oxidation of GSH at [Cu(II)] $\ll$ [GSH] does not proceed via formation of free GS[•] radicals, which is consistent also with e.s.r. investigations on other thiols [34]. It is nevertheless tempting to assume that, in the presence of DNA and under the conditions [Cu(II)]₀ > [GSH]₀ (as applied in Figures 3–5), reaction (8) is driven to the right by Cu(I) removal [reaction (2)] to form the free GS[•] species, in analogy to D[•] formation by reaction (1).

GS[•] radicals are known to oxidize NADH with a rate constant of $2.3 \times 10^8 \text{ M}^{-1} \text{ s}^{-1}$ [35] to yield NAD[•], which is further oxidized to NAD⁺ by O₂ or dimerizes to (NAD)₂ in the absence of O₂ [36].

Figure 6 Stopped-flow time profiles (a) and spectra (b) showing the slow formation of DNA-Cu(I) at $\text{GSH}/\text{Cu(II)} \geq 1$

Conditions were as in Scheme 1, but with 60 μ M CuCl_2 in solution A and 60 or 120 μ M GSH in solution B. Spectra were taken at various times after mixing with 120 μ M GSH against a blank without GSH.

Figure 7 Absorbance changes in the 300–650 nm range on interaction of Cu(II) with GSH in the absence and presence of DNA

The results were obtained by mixing (1:1, v/v) a solution containing 400 μ M CuCl_2 with a solution containing 200 μ M GSH $\pm$ 0.4 g/l DNA in 20 mM phosphate buffer (pH 7.4). A short-lived product with an absorbance peak at 360 nm, formed in both the absence (▲) and presence (○) of DNA, is detectable only during rapid mixing. The inset stopped-flow time profiles reveal that, after disappearance of the 360 nm absorbance peak (the spike is not shown), secondary products are formed which decay slowly in the presence of native DNA (a) and more quickly in the presence of heat-denatured DNA (b); no such secondary products are seen in the absence of DNA (c). The weak absorption spectrum of the secondary product in the presence of native DNA, 1 s after the stopped-flow, is also shown (□).

Using the conditions in Scheme 1, but with 50–500 μ M NADH added to solution B, the formation of DNA-Cu(I) was not affected by the additive. However, under neither aerobic nor anaerobic conditions was there any evidence for oxidation of NADH, thus it can be concluded that free $\text{GS}^\bullet$ species are not formed by the coupled reactions (8) and (2). Also DNA cleavage by GSH and Cu(II) seems not to involve $\text{GS}^\bullet$ radicals [21,22]. In the absence of GSH there was no evidence for Cu(II)-induced oxidation of NADH in the vicinity of DNA via reactions (1) and (2), as in the case of catecholamines and other bioreductive substrates [7].

GSH inhibits free-radical formation by Cu(II) in the presence of DNA

Cu(II)-induced oxidation of dopamine to yield indolic aminochrome (with absorptions around 480 nm) was previously found to be speeded up by at least two orders of magnitude on the addition of DNA. As mentioned in the Introduction, this effect could be explained [7] by reaction (2) pulling, in this case, the unfavourable redox equilibrium (10) of the dopamine-Cu(II) complex to the right (with aminochrome derived from the semiquinone $\text{RO}^\bullet$):

We have now investigated this system in the presence of GSH, by mixing (1:1, v/v) a solution containing 0.2 g/l DNA, 100 μ M CuCl_2 and 10 mM Hepes with a solution containing 50 μ M dopamine with or without 50 μ M GSH. The half-life ($t_{1/2}$) of aminochrome formation in the absence of GSH was 13 s, as previously reported [7]. In the presence of GSH, the formation of aminochrome was almost completely inhibited ($t_{1/2} > 20$ min) at the expense of DNA-Cu(I) formation by GSH, at a rate reported in Table 1 (no. 13). This result reveals that GSH can also act as an efficient antioxidant, inhibiting the generation of free radicals by Cu(II) in the vicinity of DNA via reactions (10) and (2).

Precursors of GSH-Induced DNA-Cu(I) formation

As shown in Figure 7, the interaction between Cu(II) and GSH at $[\text{Cu(II)}]_0 > [\text{GSH}]_0$ produces a short-lived product with an absorbance peak around 360 nm, in both the absence and presence of DNA. In the absence of DNA this product was only detectable during mixing, and its decay led simultaneously to secondary long-lived products with broad absorption bands below 360 nm, as indicated in Figure 4(b) (dotted curve). In the presence of DNA, the secondary products decay simultaneously with the formation of DNA-Cu(I), as mentioned above. Time profiles (Figure 7, inset) reveal, however, a striking difference in the decay of A_{360} in the presence of native (a) or denatured (b) DNA compared with the system without DNA (c). The final product, DNA-Cu(I), does not absorb at 360 nm. The decay of the 360 nm-absorbing product, however, matched well DNA-Cu(I) formation measured at 295 nm or 250 nm, for both native and denatured DNA (not shown for the conditions in Figure 7,

but see Figure 3a). The result indicates that transient ternary copper complexes are formed in the presence of DNA, involving both GSH and DNA as copper ligand. These DNA-associated transients with weak absorption above 300 nm (Figure 7) are obviously the direct precursors of DNA-Cu(I); they probably contribute also to absorbance changes around 250 nm, as mentioned above. That the precursors of the final complex are already associated with the DNA may explain why DNA-Cu(I) formation proceeds most easily when GSH interacts with Cu(II) in the vicinity of the DNA.

Formation of polyribonucleotide-Cu(I) complexes by GSH

Interaction between GSH and Cu(II) in the presence of poly(A), poly(C) or poly(I) led to characteristic absorbance changes which, in each case, could be assigned to formation of the respective Cu(I) complex, by comparison with spectra previously obtained by radiolytic reduction of Cu(II) [23]. Figure 8 shows as an example difference spectra for both GSH-induced and radiolytically induced generation of poly(C)-Cu(I). The comparison reveals that the yield is again close to 1 poly(C)-Cu(I) per GSH, under the conditions applied. Rates of formation of the Cu(I) complexes were of a similar order for DNA, poly(C) and poly(I) but much slower ($t_{1/2} \approx 90$ s) in the case of poly(A), which is consistent with the low affinity of poly(A) for Cu(I) (Figure 2).

Time profiles for GSH-induced poly(C)-Cu(I) formation were more complex than for DNA-Cu(I) formation, in that the kinetics were dependent on wavelength (Figure 8, inset): note that absorbance changes are much faster at 250 and 280 nm than at 340 nm. Wavelength-dependent kinetics were observed also for GSH-induced generation of poly(I)-Cu(I), or when ascorbate was used as reductant. One obvious reason for the wavelength-dependence is that the spectra of poly(C)-Cu(I) and poly(I)-Cu(I) change remarkably with the Cu(I)/base ratio r [8]; poly(C)-Cu(I), for instance, exhibits absorption maxima at 280 nm for $r < 0.1$, at 295 nm for $r \approx 0.3$ and at 355 nm for $r \approx 1$ (W. A. Prütz, unpublished work). Wavelength-dependent kinetics might also result from conformational rearrangements after the addition of Cu(I).

Weak absorption around 360 nm with slow decay as in Figure 7a, and assigned to precursors of DNA-Cu(I), also detectable in the case of poly(C), poly(G) and poly(I), but not for poly(U) which exhibits a very low affinity for Cu(I) (Figure 2). This provides some evidence that nucleic acid bases, and not phosphate groups, are the copper ligands of intermediates preceding the formation of nucleic acid-Cu(I) complexes.

It has already been mentioned that DNA-Cu(I) is less stable under aerobic conditions, particularly in the presence of SOD. Figure 9 shows that poly(C)-Cu(I) is also destabilized most efficiently by a combination of O_2 and SOD. We give some emphasis to this effect, as it can be explained by reductive activation of O_2 :

Although $O_2^{\cdot-}$ should be rapidly removed from the redox equilibrium (11) by reaction (12), the half-life of poly(C)-Cu(I) in the presence of SOD and O_2 was still about 14 min and that of DNA-Cu(I) under the same conditions was even of the order of 60 min. Thus reaction (11), i.e. the reverse of reactions (7) and (2), seems to be very sluggish, which is consistent with previous results showing that the O_2 -dependent catalytic activity of copper

Figure 8 Absorbance changes caused by poly(C)-Cu(I) formation in 5 mM phosphate buffer, pH 6.9

The solid spectrum was obtained 5 min after the addition of 50 μM GSH to a solution containing 50 mg/l poly(C) and 150 μM CuCl_2 under N_2 -saturated condition; inset are stopped-flow time profiles of absorbance changes in this system at the wavelength indicated. The dashed spectrum was obtained by 80 Gy γ -irradiation of an N_2O -saturated solution containing 50 mg/l poly(C), 250 μM CuCl_2 and 5 mM sodium formate, i.e. reduction of Cu(II) by 50 μM $^{\cdot}\text{CO}_2^-$ (N_2O serves to convert e_{aq}^- into $^{\cdot}\text{OH}$ [25] which enters reaction (5)).

Figure 9 Effect of O_2 and SOD on the decay of poly(C)-Cu(I) absorption at 340 nm

The results were obtained by mixing (1:1, v/v) a solution containing 0.2 g/l poly(C), 600 μM CuCl_2 and 20 mM phosphate (pH 6.8) with a solution of 50 μM GSH with (—) or without (---) 10 μM SOD. Solutions were saturated with N_2 or O_2 , as indicated.

is strongly inhibited on formation of the DNA-Cu(I) complex. Reactions (11) and (12) nevertheless provide a (slow) source of H_2O_2 which may enter the deleterious reaction (3).

Evidence for a base-paired conformation of poly(C)-Cu(I)

Figure 10(a) presents absorption and normalized fluorescence spectra of the ETB complex with poly(C), (i) in the presence of Cu(II) and (ii) after GSH-induced reduction to Cu(I). When changing from Cu(II) to Cu(I) there is a hypochromic red shift in the ETB absorption and a strong increase in the fluorescence yield. These changes closely resemble those observed when ETB intercalates into double-stranded DNA [37], thereby shielding the dye from interactions with solvent protons [38,39]. The inference from this result is that the binding of Cu(I) induces

Figure 10 (a) Absorption (—) and fluorescence (----) spectra of the ETB complex with (i) poly(C)–Cu(II) and (ii) poly(C)–Cu(I)

The results were obtained 5–10 min after preparation of air-saturated solutions containing 0.1 g/l poly(C), 100 μ M CuCl₂, 25 μ M ETB and 5 mM phosphate (pH 6.8), (i) without and (ii) with 50 μ M GSH as reductant. The fluorescence was excited at the isosbestic point (510 nm) and the intensity (*I_f*) is normalized to the peak of spectrum i. (b) Stopped-flow time profile (—) of the absorbance change at 480 nm after rapid mixing of the above system (GSH alone in one component); ····, curve obtained with 50 μ M CuCl₂ and AH⁻ (50 μ M) instead of GSH as reductant.

changes in the poly(C) conformation, enabling the intercalation of ETB. It is known that poly(C) assumes a double-stranded conformation at pH $\approx$ 4.5 by cytidine (³N) protonation and sharing of the proton with an opposite cytidine (³N), to give poly(C–H⁺)·poly(C) [40]. Cu(I) probably induces an analogous double-stranded or hair-pin conformation, i.e. poly(C–Cu⁺)·poly(C), which is stable even at pH $\approx$ 7 and into which ETB intercalates. It should be mentioned that the ETB fluorescence enhancement caused by poly(C)–Cu(I) is less than that caused by native DNA; this is probably because of quenching of the ETB fluorescence by the adjacent Cu(I), as observed also when Cu(I) was added to the DNA–ETB complex.

Stopped-flow time profiles for formation of the ETB intercalation complex with poly(C)–Cu(I) are shown in Figure 10(b). When the reduction was initiated with GSH, the kinetics of formation of the ETB complex resembled those of poly(C)–Cu(I) formation (Figure 8). The kinetics were biphasic, on the other hand, when reduction was initiated by AH⁻. Once formed, and at low ionic strength, the complex of poly(C)–Cu(I) with ETB is much more stable than poly(C)–Cu(II) itself, but the opposite behaviour was observed at high ionic strength.

In contrast with poly(C), we have not found evidence for intercalation of ETB into complexes between Cu(I) and other single-stranded polyribonucleotides such as poly(A), poly(G) and poly(U). For double-stranded poly(C)·poly(G), which in the absence of Cu(I) enables intercalation of ETB (to give spectra as in Figure 10ii), we have observed efficient inhibition of ETB intercalation on addition of Cu(I), i.e. just the opposite of that seen in the case of poly(C) alone. It is obvious from these results that extensive further investigations are required to learn more about conformational changes initiated by interactions between Cu(I) and nucleic acids.

DISCUSSION

GSH-induced reduction of Cu(II) in the presence of nucleic acids can be expected to be more complicated than that of Cu(II) reduction by ascorbate or catechols [7] via the coupled equilibria (1) and (2), because GSH itself is a strong ligand for both Cu(II) and Cu(I). In the following discussion we will try to explain some of the above observations, focus attention on Cu(I)-induced conformational changes, and address the question of pro-oxidative and/or antioxidative activity of GSH in combination

with copper and DNA, for which contradictory answers have appeared in recent literature.

Interaction of GSH with Cu(II) in the vicinity of DNA

The interactions between Cu(II) and thiols in the absence of nucleic acids, to form RS⁻–Cu(I), disulphide and other products, have been studied in some detail [41,42] and can be summarized, with GSH as an example, by reactions (13)–(16):

It must be emphasized that reaction (13) is reversible [43], thus it proceeds to the right only if Cu(I) is removed by consecutive reactions or stabilized by complexation with suitable ligands. The Cu(II)/GSH system (without DNA) proceeds towards a complicated equilibrium state [43] involving reaction (14), for which a stability constant of $1.5 \times 10^{19} \text{ M}^{-1}$ has been estimated in the case of cysteine [44]. Further oxidation of GS⁻–Cu(I) {reaction (15) [41]} and disproportionation of Cu(I) {reaction (16) [45]} are important particularly at [Cu(II)]₀ > [GSH]₀. Under aerobic conditions, Cu(I) can be expected to be removed also by reactions (17) and (18) [46], which slowly feed Cu(II) back into the catalytic cycle (at [Cu(II)]₀ < [GSH]₀ via reaction (13):

Neither the kinetics nor the equilibrium constant of reaction (13) are known. It probably proceeds via a series of intermediates depending on the [Cu(II)]₀/[GSH]₀ ratio [43,47]; the free-radical reactions (8) and (9) seem, however, not to be relevant (see above). The short-lived 360 nm absorption (Figure 7) may be mainly due to [GS⁻–Cu(II)]₂, indicating that equilibration via reaction (13) is very fast; a similar stable absorption, detected in alkaline cysteine solutions, has also been assigned to a cysteine–Cu(II) complex [48].

In the presence of DNA we tentatively consider the possibility that DNA–Cu(I) is formed via a ternary complex between

GS[−]–Cu(I) and DNA, which then interacts with Cu(II) by ligand exchange:

The formation of DNA-associated transients is indicated by the results presented in Figure 7. Direct Cu(I) transfer from GS[−]–Cu(I) to DNA is unlikely because the binding constant K_{14} is ten orders of magnitude higher than K_2 . The condition $[\text{Cu(II)}]_0 < [\text{GSH}]_0$, as applied in Figure 6, is unfavourable because Cu(II) is efficiently depleted by reactions (13)–(15) and thus not available in sufficient amount for the exchange reaction (20).

When $[\text{Cu(II)}]_0 > [\text{RSH}]_0$, an excess of Cu(II) remains available for reaction (20), which may compete favourably with reaction (15), thus enabling rapid and efficient generation of DNA–Cu(I), as shown in Figures 3 and 5. Note also that GS[−]–Cu(II) from reaction (20) feeds back into the fast equilibrium (13). When DNA is added with a delay, GS[−]–Cu(I) is removed by reactions (15) and (16), and DNA–Cu(I) is generated slowly (Figure 4), probably via the reverse reactions (16) and (15). We think that reaction (20) is the rate-determining step, as the rates increase proportionally with $[\text{Cu(II)}]_0$ (Table 1, nos. 5, 2 and 6). That the rates decrease with increasing $[\text{GSH}]_0$ (Table 1, nos. 1–3) can be roughly accounted for by depletion of Cu(II), required for reaction (20), at higher $[\text{GSH}]_0$. It is unlikely that rapid equilibration through reactions (13)–(15) produces a ‘burst’ of unbound Cu⁺ which then adds to DNA, because reaction (2) proceeds in the time scale of ms [8]. GSSG probably also plays a role as Cu(I) and Cu(II) ligand [43]; retardation of DNA–Cu(I) formation by GSSG (Table 1, no. 12) could be due to competitive binding of Cu(I) [7] or to reversal of reaction (13). O₂ seems not to interfere with the fast formation of DNA–Cu(I) under standard conditions (Table 1, no. 16), e.g. by reactions (17) and (18). On a longer time scale, reaction (11), and possibly the corresponding reaction of GS[−]–Cu(I) with O₂, could lead to reductive activation of O₂.

The above scenario, including equilibria (13) and (14) and the DNA-associated reactions (19) and (20), provides a reasonable explanation for the fast and efficient GSH-induced generation of DNA–Cu(I) in the vicinity of DNA, under conditions of $[\text{Cu(II)}]_0/[\text{GSH}]_0 > 1$. At ratios $[\text{Cu(II)}]_0/[\text{GSH}]_0 < 1$ the system becomes rather complex; it is likely in this case that the DNA accepts Cu(I) from other donors, e.g. GSSG–Cu(I) and GS[−]–Cu(I), and thus DNA–Cu(I) generation is retarded and less efficient.

Damage and protection of DNA by thiols and copper

Thiols have the reputation of being good radioprotectors [49–51], and it is commonly assumed that chemical repair of free-radical damage to biopolymers such as DNA, by hydrogen atom or electron transfer from the thiol [25,32,52,53], is one of the mechanisms important in protection. It may appear surprising therefore that thiols, including GSH, have been found to be active DNA cleavers in the presence of Cu(II), probably because of the generation of O₂-derived free radicals [20–23,54]. The literature is contradictory on this point, however, in that GSH on the other hand has been found to act as an antioxidant against the toxicological effects of copper [55], also in DNA model systems [56,57].

When scanning the above reports carefully, we recognize that copper-mediated damage to DNA in aerobic systems is activated

by thiols, particularly when $[\text{Cu(II)}]_0 > [\text{RSH}]_0$ [21,54], and is deactivated when $[\text{Cu(II)}]_0 \ll [\text{RSH}]_0$ [56,57]. Combined with the present results, we can conclude that conditions favouring the formation of DNA–Cu(I) are prerequisite to activation of oxidative damage to DNA by thiols and copper. At $[\text{Cu(II)}]_0 \ll [\text{RSH}]_0$, not only is the formation of DNA–Cu(I) impeded, as shown in this study, but Cu(I) is also stabilized by GSH towards oxidation by O₂ [55], and the thiol is likely to protect the DNA by chemical repair. That DNA cleavage by GSH and Cu(II) under the appropriate conditions is efficiently inhibited by catalase (but not by SOD) [21] provides a further strong argument in favour of reaction (3) being involved in DNA cleavage.

It may appear surprising also that DNA–Cu(I) generation by interaction of GSH with Cu(II) occurs in seconds (Figure 3d, 40 °C), whereas DNA cleavage under comparable conditions proceeds in a time scale of hours [21]. One reason is that H₂O₂ formation, via reaction (11) for instance, is sluggish (see above), but a more cogent reason is that reaction (3) is very slow. A rate constant of $k_3 \approx 20 \text{ M}^{-1} \cdot \text{s}^{-1}$ [24] has been estimated for a physiological range of pH and ionic strength. Thus if we assume, just as an example, concentrations of 10 μM H₂O₂ and 2 μM DNA-bound Cu(I), reaction (3) would proceed with a half-life of the order of 1 h. In this time scale, and under *in vivo* conditions, one would expect that H₂O₂ readily disappears from the DNA by diffusion and other reactions. Thus the question arises whether reaction (3) is really relevant to biological systems; a rather abundant source of H₂O₂ would be required to maintain its concentration in the order of the above estimate.

Furthermore, the condition $[\text{Cu(II)}] > [\text{RSH}]$ required for efficient generation of DNA–Cu(I) by thiols is also probably not biologically relevant. The content of low-molecular-mass thiols in cell nuclei certainly fluctuates during the cell cycle [58], but it is open to question whether the above condition can be achieved *in vivo*. In model systems we can assume that O₂-dependent redox cycling through reactions (13)–(14)–(15)–(17)–(18), generating H₂O₂, depletes GSH until the appropriate condition for DNA–Cu(I) formation is reached. It appears possible also that Cu(I) fixation by thiols, i.e. reaction (14) competing with reaction (2), can be masked by other metal ions. Zn(II), according to our experience, does not mask fixation of Cu(I) by GSH (Table 1, no. 10), but further studies along these lines are planned. The cytotoxicity of DTT seems actually to depend on iron rather than on copper ions [59], but iron is not as specific in initiating damage to DNA as is copper (see the Introduction).

It is interesting to note that GSH stimulates copper-mediated DNA damage in the presence of 1,10-phenanthroline [57,60] even at $[\text{Cu(II)}]_0 \ll [\text{GSH}]_0$. This is attributed to the failure of GSH to remove Cu(I) from phenanthroline. The deleterious redox activity of copper obviously depends on the Cu(I) ligands introduced: phenanthroline in combination with GSH being an activating ligand, whereas GSH alone is a deactivating ligand. This aspect may open the field for extended further research on the redox activity of copper in the vicinity of DNA.

Cu(I)-induced changes in nucleic acid conformation

As already pointed out in the Introduction, there are at least indications that Cu(I) induces a conformational B → Z transition in DNA. In this study we have shown that Cu(I) is capable also of inducing base-pairing, specifically in poly(C). Further work is certainly necessary in order to clarify such changes induced by the fixation of Cu(I). As the strength of interaction of Cu(I) depends on the base composition (Figure 2), it can be expected that Cu(I)-induced conformational changes occur preferentially in specific DNA sequences. The indications that copper plays a

role in structural organization of chromosomes are weak [2–6, 9], but we think that further attention should be paid to this possibility. If GSH were to act as an endogenous reductant initiating interactions between Cu(I) and DNA, it must again be emphasized that it may operate only in an environment, or during a phase, of low GSH concentration, or in combination with other copper ligands and metal ions.

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REFERENCES

- Bryan, S. E., Vizard, D. L., Beary, D. A., LaBiche, R. A. and Hardy, K. J. (1981) *Nucleic Acids Res.* **9**, 5811–5823
- Lewis, C. D. and Laemmli, U. (1982) *Cell* **29**, 171–181
- Yatvin, M. B., Cramp, W. A., Edwards, J. C., George, A. M. and Chapman, D. (1987) *Nucl. Instrum. Methods Phys. Res.* **A255**, 306–316
- Cramp, W. A., George, A. M., Kahn, H. and Yatvin, M. B. (1989) in *Free Radicals, Metal Ions and Biopolymers* (Beaumont, P. C., Deeble, D. J., Parsons, B. J. and Rice-Evans, C., eds), pp. 127–141, Richelieu Press, London
- Apelgot, S., Coppey, J., Grisvard, J., Guillé, E. and Sissoëff, I. (1984) *C. R. Acad. Sci. Paris* **298**, Ser. III, 31–34
- Hu, S., Fürst, P. and Hamer, D. (1990) *New Biol.* **2**, 544–555
- Prütz, W. A. (1993) *Z. Naturforsch.* **48c**, 872–878
- Prütz, W. A., Butler, J. and Land, E. J. (1990) *Int. J. Radiat. Biol.* **58**, 215–234
- Minchenkova, L. E. and Ivanov, V. I. (1967) *Biopolymers* **5**, 615–623
- Inanov, V. I., Minchenkova, L. E. and Timofeev, V. P. (1967) *Mol. Biol. (Transl.)* **1**, 572–576
- Pohl, F. M. and Jovin, T. M. (1972) *J. Mol. Biol.* **67**, 375–396
- Rich, A., Nordheim, A. and Wang, H.-J. (1984) *Annu. Rev. Biochem.* **53**, 791–846
- Masarwa, M., Cohen, H., Meyerstein, D., Hickman, D. L., Bakac, A. and Espenson, J. H. (1988) *J. Am. Chem. Soc.* **110**, 4293–4297
- Goldstein, S. and Czapski, G. (1986) *J. Free Radicals Biol. Med.* **2**, 3–11
- Stoewe, R. and Prütz, W. A. (1987) *Free Radical Biol. Med.* **3**, 97–105
- Wang, Y. and VanNess, B. (1989) *Nucleic Acids Res.* **17**, 6915–6926
- Aruoma, O. I., Halliwell, B., Gajewski, E. and Dizdaroglu, M. (1991) *Biochem. J.* **273**, 601–604
- Massie, H. R., Samis, H. V. and Baird, M. B. (1972) *Biochim. Biophys. Acta* **272**, 539–548
- Tachon, P. (1989) *Free Radical Res. Commun.* **7**, 1–10
- Reed, C. J. and Douglas, K. T. (1989) *Biochem. Biophys. Res. Commun.* **162**, 1111–1117
- Reed, C. J. and Douglas, K. T. (1991) *Biochem. J.* **275**, 601–608
- John, D. C. A. and Douglas, K. T. (1993) *Biochem. J.* **289**, 463–468
- Prütz, W. A. (1989) in *Free Radicals, Metal Ions and Biopolymers* (Beaumont, P. C., Deeble, D. J., Parsons, B. J. and Rice-Evans, C., eds.), pp. 117–126, Richelieu Press, London
- Prütz, W. A. (1990) *Z. Naturforsch.* **45c**, 1197–1206
- von Sonntag, C. (1987) in *The Chemical Basis of Radiation Biology*, chapters 3 and 10, pp. 31–56 and 295–352, Taylor and Francis, London
- Bahnemann, D., Basaga, H., Dunlop, J. R., Searle, A. J. F. and Willson, R. L. (1978) *Br. J. Cancer* **37**, Suppl. III, 16–19
- Searle, A. J. F. and Tomasi, A. (1982) *J. Inorg. Biochem.* **17**, 161–165
- Frieden, E. (1958) *Biochim. Biophys. Acta* **27**, 414–415
- Friedman, M. (1973) in *The Chemistry of the Sulfhydryl Group in Amino Acids, Peptides and Proteins*, p. 37, Pergamon Press, Oxford
- Misra, H. P. (1974) *J. Biol. Chem.* **249**, 2151–2155
- Saez, G., Thornalley, P. J., Hill, H. A. O., Hems, R. and Bannister, J. V. (1982) *Biochim. Biophys. Acta* **719**, 24–31
- Asmus, K.-D. (1983) in *Radioprotectors and Anticarcinogens* (Nygaard, O. F. and Simic, M. G., eds.), pp. 23–42, Academic Press, New York
- Prütz, W. A., Butler, J. and Land, E. J. (1994) *Biophys. Chem.* **49**, 101–111
- Davies, F. J., Gilbert, B. C., Norman, R. O. C. and Symons, M. C. R. (1983) *J. Chem. Soc., Perkin Trans. II*, 1763–1771
- Forni, L. G. and Willson, R. L. (1986) *Biochem. J.* **240**, 897–903
- Bensasson, R. V., Land, E. J. and Truscott, T. G. (1983) in *Flash Photolysis and Pulse Radiolysis*, pp. 136–140, Pergamon Press, Oxford
- LePecq, J.-B. and Paoletti, C. (1967) *J. Mol. Biol.* **27**, 87–106
- Olmsted, J. and Kearns, D. R. (1977) *Biochemistry* **16**, 3647–3654
- Porumb, H. (1978) *Prog. Biophys. Mol. Biol.* **34**, 175–195
- Guschlbauer, W. (1976) in *Nucleic Acid Structure*, pp. 70–73, Springer Verlag, New York
- Kolthoff, I. M. and Stricks, W. (1951) *J. Am. Chem. Soc.* **73**, 1728–1733
- Klotz, I. M., Cerlinski, G. H. and Fiess, H. A. (1958) *J. Am. Chem. Soc.* **80**, 2920–2923
- Hemmerich, P. (1966) in *The Biochemistry of Copper* (Peisach, J., Aisen, P. and Blumberg, W. E., eds.), pp. 15–32, Academic Press, New York
- Stricks, W. and Kolthoff, I. M. (1951) *J. Am. Chem. Soc.* **73**, 1723–1727
- Espenson, J. H., Shaw, K. and Parker, O. J. (1967) *J. Am. Chem. Soc.* **89**, 5730–5731
- Zuberbühler, A. (1970) *Helv. Chim. Acta* **53**, 473–485
- Leu, A.-D. and Armstrong, D. A. (1986) *J. Phys. Chem.* **90**, 1449–1454
- Cavallini, D., de Marco, C., Dupre, S. and Rotilio, G. (1969) *Arch. Biochem. Biophys.* **130**, 354–361
- Hagen, U. and Koch, R. (1957) *Z. Naturforsch.* **12b**, 240–249
- Foye, W. O. (1973) *Int. J. Sulfur Chem.* **8**, 161–171
- Livesey, J. C. and Reed, D. J. (1987) *Adv. Radiat. Biol.* **13**, 285–353
- Adams, G. E. (1972) *Adv. Radiat. Chem.* **3**, 125–208
- Liphard, M., Bothe, E. and Schulte-Frohlinde, D. (1990) *Int. J. Radiat. Biol.* **58**, 589–602
- Rosenkranz, H. S. and Rosenkranz, S. (1971) *Arch. Biochem. Biophys.* **146**, 483–487
- Hanna, P. M. and Mason, R. P. (1992) *Arch. Biochem. Biophys.* **295**, 205–213
- Chiou, S.-H. (1983) *J. Biochem. (Japan)* **94**, 1259–1267
- Milne, L., Nicolera, P., Orrenius, S. and Burkitt, M. J. (1993) *Arch. Biochem. Biophys.* **304**, 102–109
- Jocelyn, P. C. (1972) in *Biochemistry of the SH Group*, pp. 230–231, Academic Press, London
- Held, K. D. and Melder, D. C. (1987) *Radiat. Res.* **112**, 544–554
- Que, B. G., Downey, K. M. and So, A. G. (1980) *Biochemistry* **19**, 5987–5991