ELSEVIER

Journal of Photochemistry and Photobiology A: Chemistry 142 (2001) 9–18

Journal of Photochemistry and Photobiology A:Chemistry

www.elsevier.com/locate/jphotochem

Role of iron ions in damage to DNA: influence of ionising radiation, UV light and H_2O_2

Hanna B. Ambroz [a,*], Tony K. Bradshaw [b], Terence J. Kemp [c], Ewa M. Kornacka [a], Grazyna K. Przybytniak [a]

[a] *Institute of Nuclear Chemistry and Technology, ul. Dorodna 16, 03-195 Warszawa, Poland*
[b] *School of Biological and Molecular Sciences, Oxford Brookes University, Oxford OX3 0BP, UK*
[c] *Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK*

Received 11 February 2001; received in revised form 2 April 2001; accepted 10 April 2001

Abstract

Addition of Fe(III) increases the number of strand breaks of plasmid DNA in aqueous solution under gamma-radiolysis at room temperature and at 77 K. Low temperature radiolysis requires ca. 100 times higher radiation dose for the creation of comparable strand breaks due to the suppression of Fenton-type reactions. Room temperature radiolysis produces a relatively large number of multi-single strand breaks (ssb) and, on absorption of 100 Gy, less double-strand breaks (dsb), ca. 4%, while under cryogenic conditions at a dose of 10 kGy, are produced ca. 10% dsb for a similar total number of strand breaks. Fe(III) in its complex with EDTA exhibits small, but distinct damage to DNA even without irradiation, in comparison to the strong effect of Fe(II)/EDTA. Under our experimental conditions hydrogen peroxide does not influence the damage in a noticeable way in the presence of Fe(II) and Fe(III), although UV light exhibits a very strong effect on the addition of both Fe(III) and H_2O_2. In our system, iron forms complexes with EDTA and also is bound by other components. A molar excess of iron in relation to EDTA has no striking effect. The chelators seem to be responsible for creation of a reactive form of iron, able to produce reactive oxygen species in solutions containing dissolved air.

Keywords: DNA damage; EDTA; Iron ions; Fenton-type reactions; Radiolysis; Strand breakage

1. Introduction

In our previous paper, we have reported EPR studies of the effects of iron and copper ions on damage to DNA by ionising radiation at cryogenic temperatures [1]. Under these conditions, the ions exhibit a protective influence in lowering the total level of DNA radicals generated, as recorded previously [1–3]. The effect is stronger at increased concentrations of both metal ions. At cryogenic temperatures, Fenton-type reactions have no possibility to develop and can proceed only as secondary processes at increased temperatures. We have observed selective loss of the $TH^{\bullet}$ radical at ca. 203 K in the presence of Fe(III), pointing to electron scavenging by iron localised close to thymine base.

We now wish to report complementary studies of the influence of iron as examined by gel electrophoresis using plasmid DNA for estimation of single and double strand breaks (ssb and dsb). Transition metal irons play a much stronger role in all room temperature experiments, and a weaker effect in post-effect reactions if radiolysis is carried out under cryogenic conditions. Under irradiation, cellular DNA is damaged both by the direct (due to direct ionisation) and indirect effects, the latter being due to attack on DNA by reactive species, mainly e_{aq}^- and $^{\bullet}OH$ radicals produced in the aqueous environment [4,5]. The role of iron is very important for all biological systems as it is present in cell nuclei and plays crucial roles in the biochemistry of oxygen, in both its positive or damaging reactivity [6,7]. Despite the enormous amount of work done in this field, some mechanisms are still considered controversial or obscure. The book recently published by Symons and Gutteridge on this topic summarises the importance and complexity of the problem from the physicochemical, biochemical and medical viewpoints [8]. The most active oxidation state of iron ions producing hazardous oxidising species in biosystems remains uncertain, candidates being Fe(II) or Fe(IV). Ferrate anion $Fe(VI)O_4^{2-}$ has also been identified as a powerful oxidising agent in aqueous solution [8,9]. According

Abbreviations: EDTA: ethylenediaminetetraacetic acid; HEDTA: *N*-(hydroxyethyl)ethylenediaminetriacetic acid

*Corresponding author. Tel.: +48-22-811-23-47; fax: +48-22-811-15-32.
E-mail address: ambroz@orange.ichtj.waw.pl (H.B. Ambroz).

1010-6030/01/$ – see front matter
PII: S1010-6030(01)00439-7

to Symons, the Fe(II)/O_2 complex is better described as the 'closed-shell' species Fe(IV)/O_2^{2-} [8]. Fe(III) is commonly accepted as less dangerous, although it can be easily bound by ligands and some of them are claimed to be able to change its oxidation state [10,11]. Generally, ligands with oxygen donors are considered to bind preferably to Fe(III) (e.g. desferrioxamine) and those with nitrogen donors to Fe(II) (e.g. 1,10-phenanthroline). EDTA which contains oxygen and nitrogen atoms is a good ligand for Fe(II) and Fe(III). EDTA as a ligand is known to stimulate the autoxidation of Fe(II) to Fe(III), while not being known to reduce Fe(III).

However Fe/EDTA complexes can react in very different ways, sometimes even opposite, to accelerate or inhibit some reactions depending on conditions, e.g. on the Fe:EDTA ratio [8,11–13]. Some of these problems we refer to below in discussion.

2. Experimental

Plasmid pBR322 in EDTA-Tris buffer, pH = 7.6, was obtained from MBI Fermentas (Tris: tris(hydroxymethyl)-aminomethane $(HOCH_2)_3CNH_2$). Aqueous solutions were prepared with de-ionised doubly distilled water. Other chemicals were supplied as follows: $FeCl_3$ (Aldrich), $FeCl_2{\cdot}4H_2O$ (Avocado Research Chemicals), H_2O_2 (30%, Merck). DNA solutions prepared for chemically induced damage after addition of Fe(III) or Fe(II) ions, were kept for 24 h at 4°C, and then loaded with H_2O_2 (or diluted by the same volume of water). Other samples were irradiated with a ^{60}Co source at room temperature or at 77 K in the presence or absence of Fe(III) ions, as indicated in the figures. Photolyses were carried out with a Bausch and Lomb 150 W point-source xenon lamp (full output, parallel beam, samples located 30 cm from lamp).

The irradiated samples consisted of the following components: 0.6 mM nucleotides, 0.4 mM EDTA, 4 mM Tris, and $FeCl_3$ in the range of concentrations 0–1 mM. A series of unirradiated samples, except $FeCl_2$ or $FeCl_3$ in buffer, also included H_2O_2 from 0 to 100 mM. Before electrophoresis the sample was mixed with gel loading buffer which contained Ficoll, bromophenol blue and xylene cyanol FF. In order to perform electrophoresis the gel was prepared from agarose (Serva) and Tris-acetate-EDTA (TAE) buffer, pH 8.0. The mixture was heated until all agarose was dissolved and then, while the agarose solution was cooling, ethidium bromide was added to a final concentration of 0.5 μg/ml. The gel was poured into the casting tray, cooled to room temperature and placed in the electrophoresis apparatus. The electrophoresis was carried out for 2 h under TAE running buffer at a voltage of 100 V. Afterwards the gel was illuminated with a UV transilluminator TEC and the results, recorded with a digital camera, were saved in a computer and examined applying DNAnalysis software [14]. The program allows for determination of the percentage of supercoiled, relaxed and linear forms of plasmid separated by gel electrophoresis. A coefficient of 1.5 was applied to supercoiled plasmid which binds less ethidium bromide due to the compact configuration of the undamaged plasmid molecule. It was found that the commercially-supplied DNA showed a single-strand breakage figure of between 11 and 18% on electrophoresis, a presence evident in our data for blank samples in the figures.

3. Results and discussion

3.1. Radiolysis at room temperature and under cryogenic conditions

The influence of Fe(III) on single and double strand breaks (ssb and dsb) of plasmid DNA in aqueous solution following radiolysis at room temperature is illustrated in Figs. 1 and 2, versus concentration of Fe(III) from 0 to 1 mM (Fig. 1) and versus absorbed dose from 0 to 200 Gy (Fig. 2). The experiments for higher concentrations of Fe(III) (3 and 5 mM) are not shown as the results were poorly reproducible, mostly because the plasmid stayed in wells during electrophoresis due to conformational changes of DNA.

The set of diagrams in Fig. 1 show that a distinct effect of Fe(III) can be observed even in the absence of radiation. Generally, within the range of absorbed doses, the influence of iron is not very strong as can be seen from the slopes of the lines. Without irradiation and at 10 Gy dose, only ssb damage is detected, while at and above 50 Gy dsb is also generated. The dependence of ssb on the absorbed dose (Fig. 2) is linear at low doses while above 50 Gy it reaches a plateau at the point, where dsb becomes apparent. At 1 mM Fe(III) concentration the gradient of the curve corresponding to singly damaged plasmid becomes negative at doses above 100 Gy and dsb is formed via already damaged plasmid (ssb line). This suggests that some secondary breaks of ssb-type to the plasmid molecule are formed on an opposite strand sufficiently close to the first single break to create dsb.

Statistical treatment enables calculation of the average numbers of ssb and dsb per plasmid. Absorption of 100 Gy generates 1.16 ssb per plasmid at 0 mM Fe(III) and 2.04 ssb/plasmid at 1 mM Fe(III) (Fig. 3). In these cases, ca. 30% and 4% of supercoiled DNA is found, respectively in the gel following electrophoresis, which means that at 100 Gy there is ca. one-third of undamaged plasmid at an average of 1.16 ssb/plasmid. This reflects a mechanism of break formation such that, at this dose, multi-single strand breaks are relatively common. As chemical changes in gamma-irradiated aqueous systems proceed mainly due to the indirect effect, the energy of the radiation is absorbed mostly in water and the reactive species so created are responsible for starting and developing Fenton-type processes at room temperature. The dsb distribution per molecule [15] corresponds closely to the amount of detected linear form of the plasmid: 0.048 dsb/plasmid and 5.4% of linear molecules at zero Fe(III) and ca. 0.083 dsb/plasmid, and 8.3% of linear form at 1 mM concentration of Fe(III). In

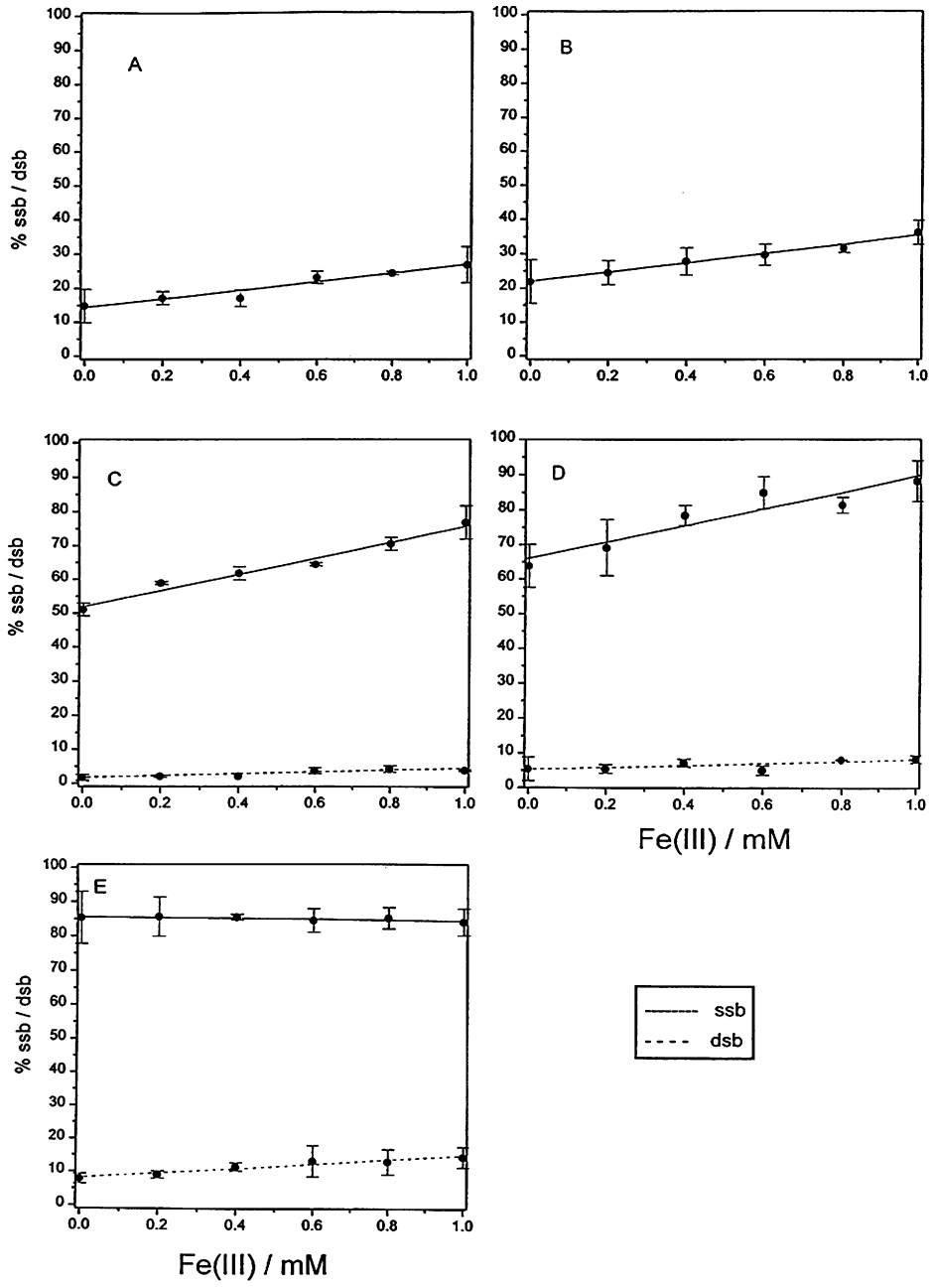


Fig. 1. The influence of Fe(III) on DNA strand breaks following doses of (A) 0 Gy, (B) 10 Gy, (C) 50 Gy, (D) 100 Gy, and (E) 200 Gy, at room temperature.

the calculation, all necessary corrections are considered, including that which refers to plasmid containing originally <100% of supercoiled form.

Experiments with radiolysis performed at 77 K required doses of radiation higher by a factor of 100 to obtain comparable numbers of breaks as reported previously without addition of iron [16]. The results are depicted in Figs. 4 and 5, as functions of Fe(III) concentration (Fig. 4) and of absorbed dose, respectively (Fig. 5). As the procedures remained the same for all treatments, the standard deviations are similar, but are not displayed for reasons of clarity.

Increased concentrations of Fe(III) influence the formation of ssb and dsb, but the effects are not very strong. The characteristic non-linear behaviour appears at absorbed doses above 5 kGy and at Fe(III) above 0.2 mM when supercoiled plasmid decays and dsb is generated on account of singly broken molecules. As can be seen in Fig. 5, between doses of 1 and 5 kGy, the increase in ssb is striking, while dsb formation appears to be linear over the whole range examined within the error of the method. Statistical calculations of the number of breaks per plasmid on absorption of 10 kGy at 77 K show comparable damage as for absorption

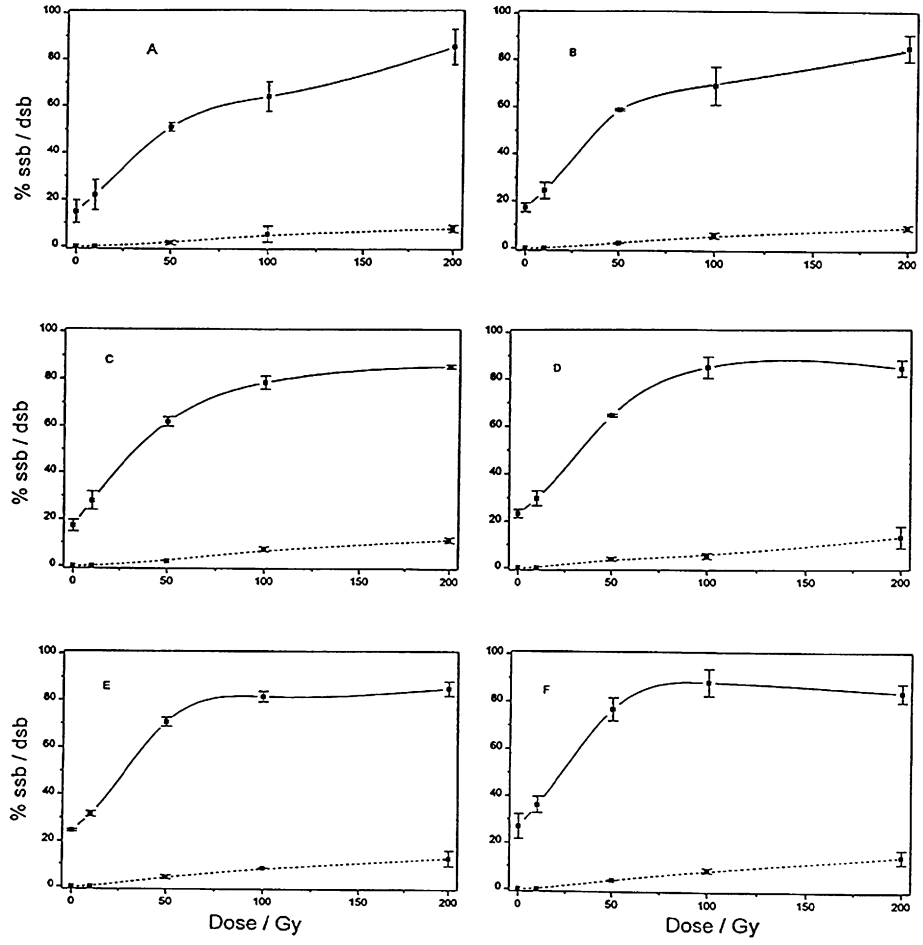


Fig. 2. The influence of γ-irradiation doses on DNA strand breaks in presence of Fe(III) at concentrations of (A) 0 mM, (B) 0.2 mM, (C) 0.4 mM, (D) 0.6 mM, (E) 0.8 mM, and (F) 1.0 mM, at room temperature. Full line, ssb; broken line, dsb.

of 100 Gy in room temperature radiolysis, with a linear increase in the ssb and dsb/plasmid up to 0.8 mM Fe(III) (Fig. 6). It can be estimated from the growth of both types of breaks under the experimental conditions, that the lower temperature leads to relatively more double strand breaks. The results with Fe(III) exceeding 0.8 mM were not valid for statistical treatment as the population of the linear form of the plasmid exceeded 0.1 (10%).

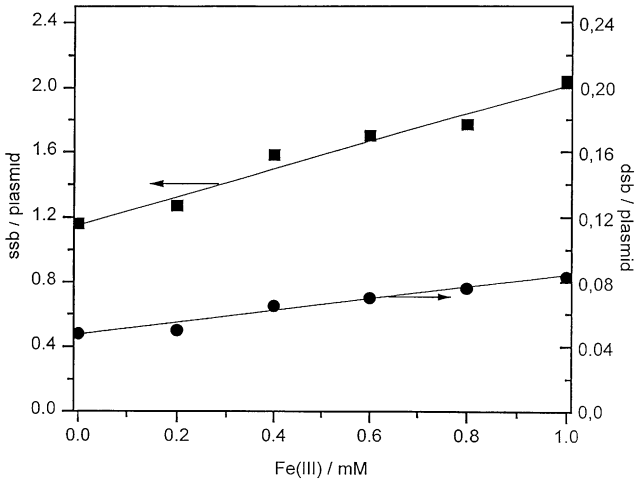


Fig. 3. The number of ssb per plasmid and dsb per plasmid as a function of Fe(III) concentration upon a dose of 100 Gy at room temperature.

Under irradiation at 77 K, most direct damage is randomly inflicted on DNA as diffusion has largely ceased. This means that the predominant effect on the addition of Fe(III) is protective, due to the scavenging of electrons by Fe(III), reducing it to Fe(II). As is known from EPR studies, all radicals created in the ice phase cannot cross the phase barrier and recombine there at elevated temperatures without increasing the population of DNA radicals [1]. Only molecular species are sufficiently long-lived to react at elevated temperatures. On warming, some Fenton-like reactions develop in the system as reactive species, e.g. H_2O_2 originating from recombination of OH radicals, become mobile and are close to reduced iron within a reaction radius. This explains the lower overall populations of single and double breaks of DNA strands and the requirement of ca. 100-fold higher doses at 77 K for damage comparable to that found on room temperature radiolysis. This results in a lowering of the effect of

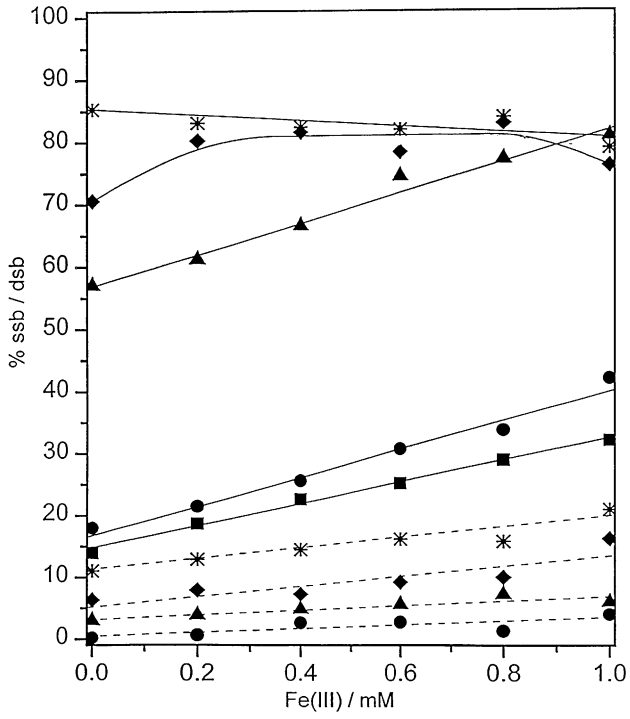


Fig. 4. Dependence of ssb (—) and dsb (- - -) on the Fe(III) concentration on various irradiation doses at 77 K (■) 0 kGy, (●) 1 kGy, (▲) 5 kGy, (◆) 10 kGy, (✱) 20 kGy.

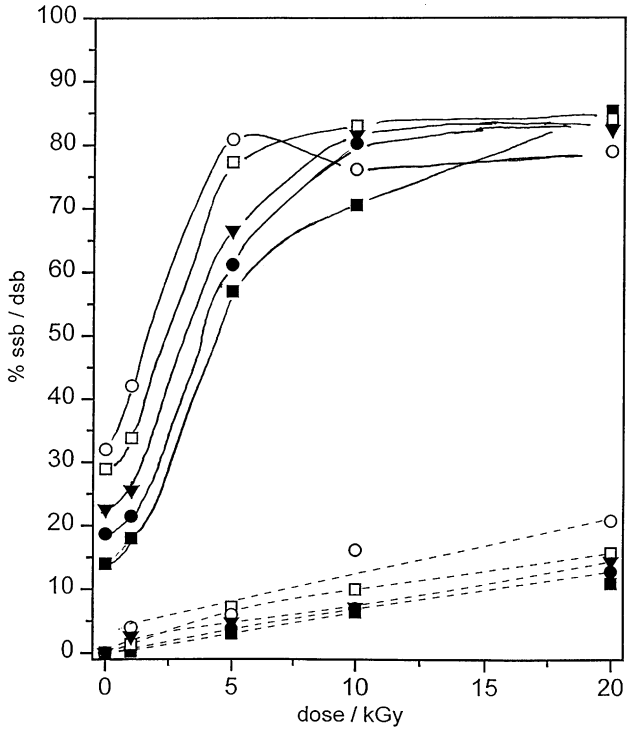


Fig. 5. Dependence of ssb (—) and dsb (- - -) for increasing concentrations of Fe(III) (■) 0 mM, (●) 0.2 mM, (▼) 0.4 mM, (□) 0.6 mM, (○) 1.0 mM on irradiation at 77 K.

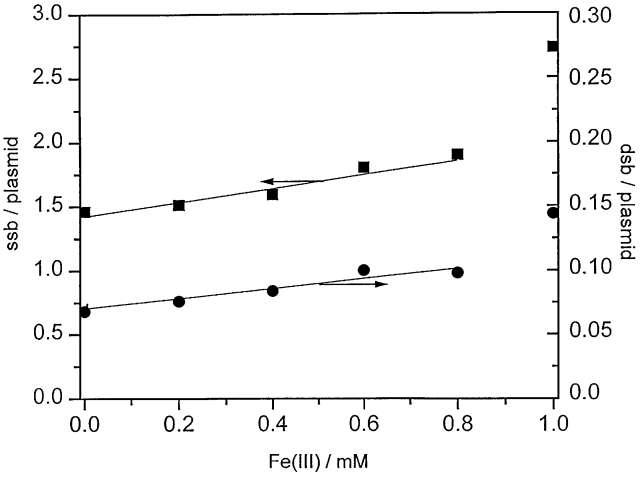


Fig. 6. Numbers of ssb per plasmid and dsb per plasmid as a function of Fe(III) concentration on a dose of 10 kGy at 77 K.

iron under cryogenic conditions (Figs. 3 and 6). The role of iron is much more effective when Fenton-type reactions can proceed freely under irradiation. The ratio of single/double strand breaks is more than 2-fold higher at room temperature, and equals ca. 25, in comparison with the value of ca. 9.5 at 77 K under the experimental conditions. This means that dsb are formed more readily via the direct effect of ionising radiation on DNA molecules, while the indirect effect creates more ssb. Similar studies on irradiation of aqueous plasmid without iron gave ssb/dsb ratios of 21 and 15, respectively [16]. We believe that our results define the role of iron in promoting ssb at room temperature and dsb at 77 K.

3.2. Treatment with H_2O_2

These experiments were performed to elucidate some aspects of the influence of iron on damage to DNA in the absence of ionising radiation, which were done as blank samples. Low concentrations of iron at the catalytic level (up to 0.24 mM) and a large excess of hydrogen peroxide (up to 5 mM) were utilised to follow Fenton-type reactions.

Under our conditions only a weak influence of Fe(III) in enhancing ssb can be observed even at high hydrogen peroxide concentration (Fig. 7). Note the 18% ssb is intrinsic to the original sample of DNA. The effect of an increased amount of H_2O_2 is also not very appreciable: it is stronger at low concentration and seems to saturate at 1 mM, indicating an inhibition of the catalytic chain reactions (Fig. 8).

At the same molar concentration as Fe(III), Fe(II) instigates a sharp increase in single breaks but with a still limited effect of added H_2O_2 (Figs. 9 and 10). At ca. 0.15 mM concentration, Fe(II) on its own generates over 50% singly broken form of plasmid under the applied conditions. No dsb was observed even when almost no supercoiled form of the plasmid was left.

No noticeable influence of Fe(III) was detected on increasing the damage in the presence of hydrogen peroxide,

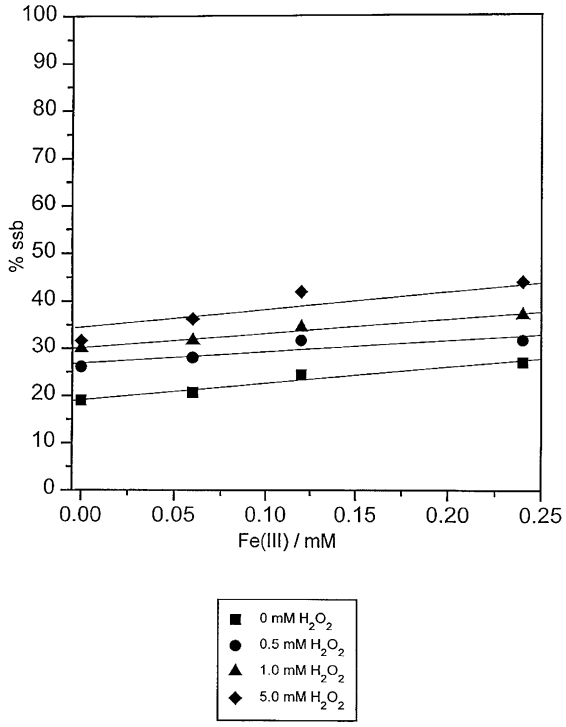


Fig. 7. Effect of Fe(III) on strand breakage at various concentrations of H_2O_2, room temperature.

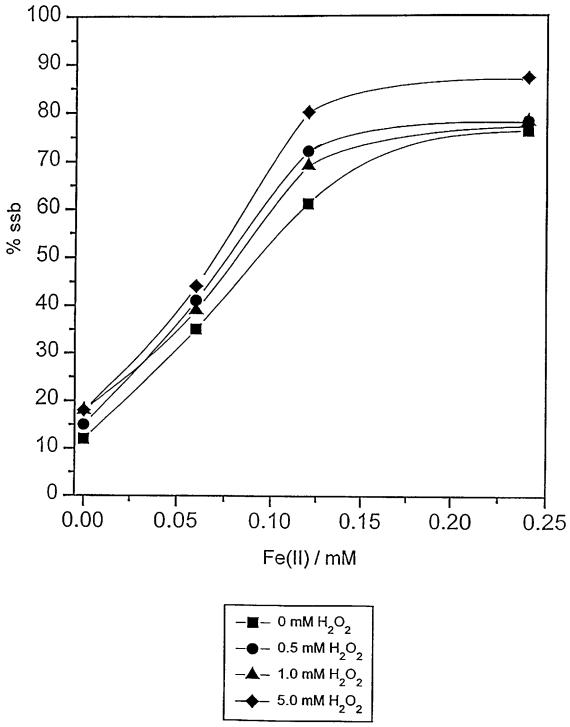


Fig. 9. Effect of Fe(II) on strand breakage at various concentrations of H_2O_2, room temperature.

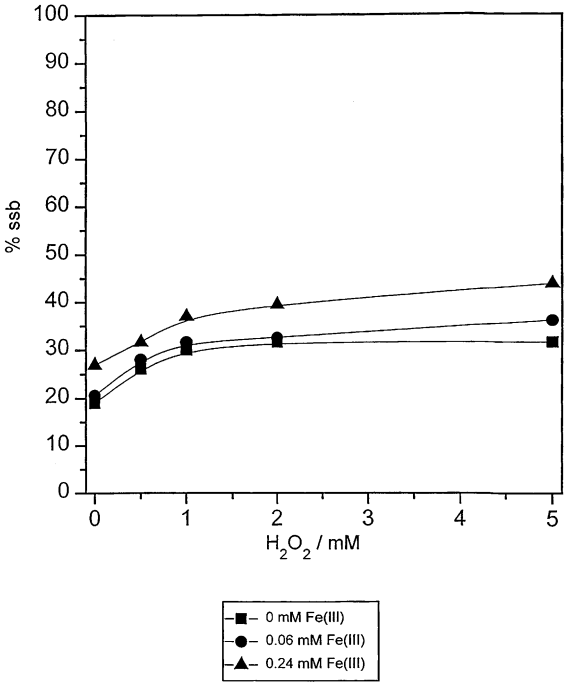


Fig. 8. Effect of H_2O_2 on strand breakage at various concentrations of Fe(III), room temperature.

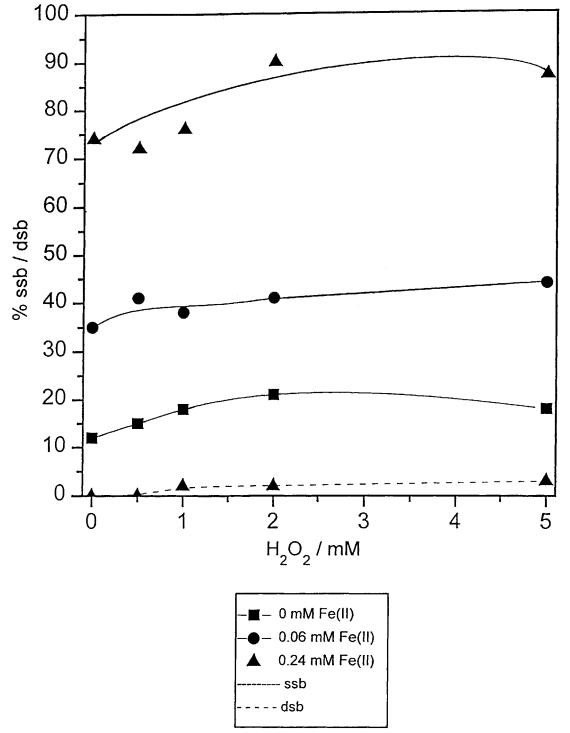


Fig. 10. Effect of H_2O_2 on strand breakage at various concentrations of Fe(II), room temperature.

in contrast to the effect of Fe(II) alone, which seems to confirm that we do not observe efficient reduction of Fe(III) by HO_2^-. The reduction of iron by peroxide anion (pK_a of H_2O_2 = ca. 12) does not seem to be able to compete with another mechanism of reactive iron formation, e.g. via the reduction by EDTA under our conditions. The reason could be steric, i.e. difficulty of access of the HO_2^- anion to iron in its complexes. If Fe(III) was reduced by HO_2^- to Fe(II), then on increasing the concentration of H_2O_2, more additional breaks should be observed (Figs. 7 and 8).

3.3. Effect of UV light

Another situation is observed under UV light, namely the instigation of Fenton-type reactions of iron and hydrogen peroxide to produce hydroxyl radicals (Figs. 11 and 12). At short-time photolysis, small additions of Fe(III) and hydrogen peroxide induce great enhancement of ssb. The characteristic features of the Fenton-type reactions are evident — the presence of a single component, either iron or hydrogen peroxide, causes a much smaller effect than both of them together, thus, we found a dramatic increase in ssb formation on their joint addition. These conditions also induce dsb which requires two ssb located within up to ca. 15 base pairs on opposite strands. In our experiments such dsb must originate from intensive attack of damaging agents

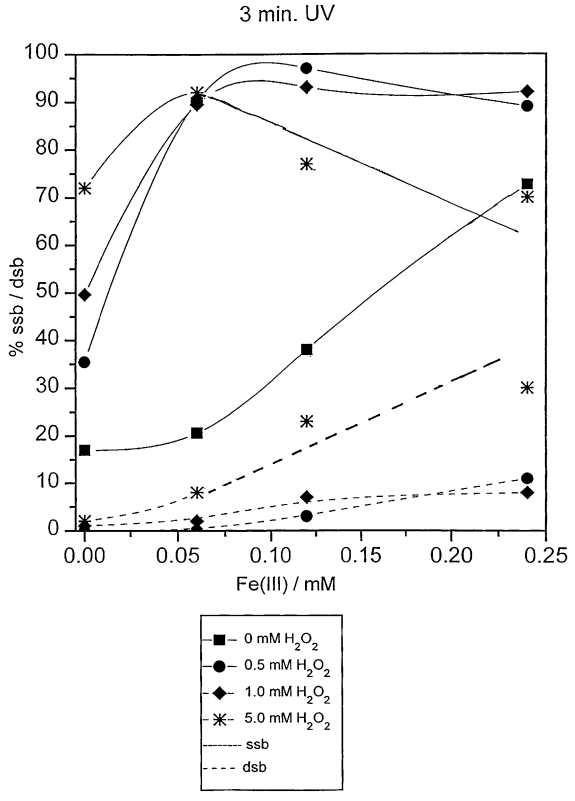


Fig. 11. Influence of Fe(III) at various concentrations of H_2O_2 on formation of ssb and dsb on UV photolysis.

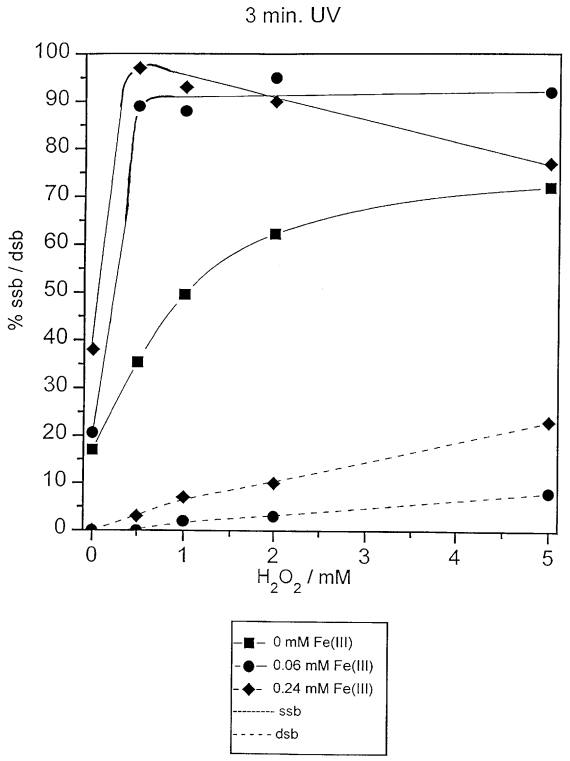


Fig. 12. Influence of H_2O_2 at various concentrations of Fe(III) on formation of ssb and dsb on UV photolysis.

to already singly-broken strands. The hydroxyl radical is an extremely reactive species and reacts non-specifically at the site where it is generated, i.e. it should be not more than a few nanometers from DNA. This suggests that either $^{\bullet}OH$ radicals are produced extensively or other secondary reactive species are involved [17].

The direct effect of UV light in effecting break formation has to be excluded, the 18% of ssb being intrinsic to the original DNA sample, and any damage ascribed to the catalytic processes of iron/hydrogen peroxide, as can be seen from Figs. 7 and 8 (no UV) and Figs. 11 and 12 (with UV). The blank samples without any addition of iron and H_2O_2 show a similar amount of ssb under the conditions of photolysis. The photo-induced reduction of hydrated Fe(III) via electron transfer from a water molecule should be negligible in the complex Fe/EDTA/H_2O:

$$\mathrm{Fe(III)} \cdot n\mathrm{H_2O} + h\nu(230\,\mathrm{nm}) \rightarrow \mathrm{Fe(III)} \cdot (n-1)\mathrm{H_2O} + \mathrm{H_2O^{\bullet +}}$$

All these experiments demonstrate that neither an increased concentration of Fe(III) nor of H_2O_2 noticeably accelerates damage without the influence of light.

3.4. Iron ions and chelators

Our results indicate that damage to DNA, as ssb, is caused by iron itself in the system, in the absence either of irradiation or hydrogen peroxide. The effect of increased

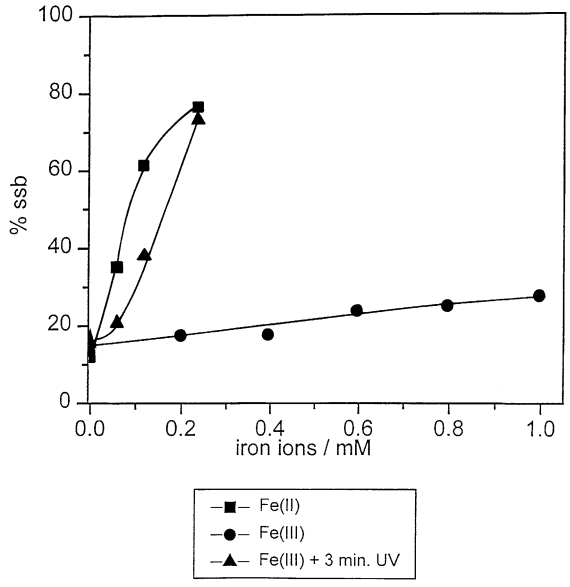
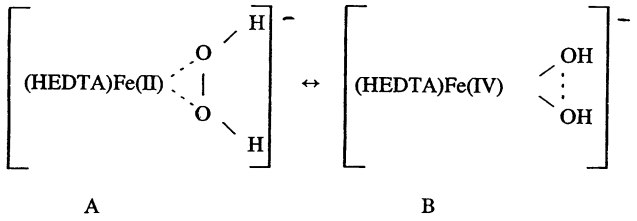


Fig. 13. Percentage of ssb as a function of Fe(II) and Fe(III) (under UV light and without UV).

concentrations of Fe(II) and Fe(III) alone on ssb is illustrated in Fig. 13, together with data following short photolysis with UV light (for clarity, the data are collected from Figs. 1a, 4, 7, 9 and 11). Fe(II) appears to exert a powerful influence on ssb formation, while Fe(III) has a weak effect, which suggests that reduced iron might be responsible for the damage in both cases. The curve of ssb versus concentration of Fe(III) could be then controlled by the reduction of Fe(III) to Fe(II), and efficient $^{\bullet}$OH radical formation would proceed in the Fe(II)/EDTA/H_2O system in oxygenated aqueous solutions as found earlier [12,13,18,19].

If this is the case in our system, there must be a process able to initiate chain reactions by electron transfer to Fe(III) without any additional applied stimulus. The most probable reducing species would be EDTA, which forms stable ferric and ferrous complexes and is commonly applied in studies of iron-catalysed reactions and also used clinically. Von Sonntag and Hoebel found in pulse radiolysis studies that the *N*-centred radical cation of EDTA can be formed at neutral pH with an attributed pronounced optical absorption at 480 nm [20]. The reaction depends on the protonation of nitrogen. At neutral pH, EDTA is present mostly in the tri-anionic form and nitrogen is unlikely to be protonated. However, we cannot completely exclude other components, such as Tris and DNA bases containing nitrogen atoms as good electron donors, and DNA can also bind iron ions at its phosphate groups as it is negatively charged at neutral pH.

EDTA coordinates Fe(III) and Fe(II) via oxygen and nitrogen to form water-soluble complexes, preventing precipitation of iron as polynuclear ferric oxidohydroxides. Fe(II)/EDTA inhibits or stimulates oxidation reactions, depending on the molar ratio Fe:EDTA, pH or even on the method of preparation of the complex [8]. Despite its



Scheme 1.

complex nature, EDTA is considered to promote the aerial oxidation of Fe(II) to Fe(III) at neutral pH [8,11]. There are no available data on the direct thermal reduction of Fe(III) by EDTA, although Fe(III)/EDTA in presence of oxygen was found to promote oxidation of thiols and phenols [21]. Generally, in such experiments, not more than one chelate is utilised and no data are presented relating to the distribution of iron between different ligands [10,11]. It seems that in such an equilibrium, iron should be bound by various ligands in a way strongly dependent on conditions. The only qualitative information about chelators being good or bad for Fe(III) or Fe(II), binding them quickly or slowly, seems incomplete, and if a system is not equilibrated, some adventitious species could be involved. The oxidation state of iron in particular complexes is also ambiguous, as some authors postulate that Fe(II) quickly oxidised to Fe(III) on addition of a selectively-binding Fe(III) chelator, and vice versa, i.e. Fe(III) is reduced to Fe(II) in the presence of Fe(II) chelators [10].

In some reactions systems at low pH, Fe(III) complexed with polyamino-carboxylic acids in dimeric form was presented as a resonance form of Fe(II) and Fe(IV) complexes (Scheme 1) [9]. Based on thermodynamic calculations, the authors suggest that the ferryl Fe(IV) complexes may constitute the major pathway under biological conditions at neutral pH with EDTA-like ligands [22]. If we accept this mechanism in our case, then both Fe(II) and Fe(IV) can be responsible for damage to plasmid DNA. Later this was questioned and it was suggested that the first step in this process was the formation of a transient complex $L_m/M/H_2O_2{}^{n+}$ which might decompose to an $^{\bullet}$OH radical or a higher oxidation state of the metal $L_mM^{(n+2)+}$ [23]. These problems are still under discussion. In the case of iron it would be Fe(II) and Fe(IV). Whatever is the mechanism, complexed Fe(II) and Fe(III) themselves, in the presence of dissolved oxygen, are able to destroy plasmid DNA, respectively, more or less efficiently.

It seems reasonable to accept that iron in such complexes shares electrons with its ligands and, depending on their character and conditions, various iron ions can be available in a form damaging to biomolecules.

Fe(III) is known to be reduced photolytically by many organic anions, e.g. the reaction with oxalic acid anion is applied in actinometry [24]. Our results clearly show that the Fe(III)/EDTA/H_2O complex is photosensitive. Qualitative experiments with aqueous solutions of Fe(III)/EDTA

and Fe(III)/EDTA/Tris exhibit a gradual lowering of the optical absorption of the solutions in the laboratory under day-light (October, cloudy day) with different kinetics for both systems. The processes are accelerated by more intense irradiation of the samples. We observed negligible changes of pH on addition of $FeCl_3$ to EDTA/Tris at concentrations of Fe(III):EDTA equal to 2:1 and a 10-fold excess of Tris.

As in our system there must be a competition in binding iron between DNA and EDTA/Tris, and at equilibrium the iron distribution is probably the reason why we do not observe any dramatic changes at the point when the molar concentration of iron exceeds that of EDTA, as observed previously [8,25]. The equilibrium process has to take time and different results are observed, depending on the Fe:EDTA ratio, varying from stimulation to inhibition, which could originate from the fact that under particular conditions there were available different amounts of 'free' iron ions not complexed with EDTA. In our system it is probable that there are no 'free' iron ions, despite the lower molar concentration of EDTA in relation to iron.

Short UV photolysis increases greatly the effect of Fe(III) on ssb formation. It seems that production of 'reactive' iron, e.g. via electron transfer from EDTA to iron, proceeds much faster when the complex is excited. UV light also accelerates the homolysis of hydrogen peroxide, and an increase in the hydroxyl radical concentration causes extensive damage (Figs. 11 and 12). The effect is purely due to indirect damage to DNA as blank experiments show that at 0 mM of Fe(III) and 0 mM of H_2O_2, UV light causes negligible ssb (see above), the 18% level of ssb in the blank sample being intrinsic to the original sample of DNA.

3.5. Reactive oxygen species

Studies on the roles of iron in damaging DNA or other biological systems are commonly performed under conditions when reactive oxygen species as $^{\bullet}OH$, $HO_2^{\bullet}$, e_{aq}^{-} or $O_2^{-\bullet}$ are present in the systems due to ionising radiation, photolysis or on addition of hydrogen peroxide. We observe damage to DNA even without these instigations. Moreover, we have found an unexpectedly small effect of the addition of hydrogen peroxide without UV light, both in the Fe(III) and Fe(II) systems. The most damaging species in our cases, without any radiation or UV-excitation, appears to be reduced iron in the presence of dissolved oxygen in solution. Further experiments are needed to clarify this point.

The mechanism of Fe(II) damage to DNA can be based on its reaction with oxygen dissolved in aqueous solution. Some authors postulate that reduced iron slowly reacts with oxygen to form superoxide radical in a reversible process [19]:

$$Fe(II) + O_2 \rightleftharpoons Fe(III) + O_2^{\bullet -}$$

Superoxide radical undergoes further fast reactions to form hydroxyl radical and then the Fenton-type reactions can proceed:

$$2O_2^{\bullet -} + 2H^+ \rightleftharpoons 2HO_2^{\bullet} \rightarrow H_2O_2 + O_2$$

$$H_2O_2 + Fe(II) \rightarrow OH^{\bullet} + OH^- + Fe(III)$$

According to this mechanism, the reactive oxygen species produced are able to break the DNA backbone via the degradation of the sugar moiety. If iron is bound by DNA bases, another site-specific reaction can take place at the sugar.

Some authors disclaim the involvement of free superoxide ion and suggest another sequence of reactions leading to H_2O_2 formation [12,18] (L = EDTA in various states of protonation):

$$LFe^{II}(H_2O) + O_2 \rightarrow LFe^{II}(O_2) + H_2O$$

$$LFe^{II}(O_2) \rightarrow LFe^{III}(O_2^{-\bullet})$$

$$LFe^{III}(O_2^{-\bullet}) + LFe^{II}(H_2O) \rightarrow LFe^{III}(O_2^{2-})Fe^{III}L + H_2O$$

$$LFe^{III}(O_2^{2-})Fe^{III}L + 2H_2O + 2H^+ \rightarrow 2LFe^{III}(H_2O)H_2O_2$$

Fe(II) and the hydrogen peroxide generated in the system can then start Fenton-type chain reactions [18].

Acknowledgements

The authors express their thanks to Professors Martyn Symons and Clemens von Sonntag for their helpful comments. They also thank Prof. I. Szumiel, Dr. M. Kruszewski, Dr. I. Gradzka and Dr. N. Jaroszewicz for their consultations, help and assistance with all biological aspects of this work.

References

[1] H.B. Ambroz, T.J. Kemp, E.M. Kornacka, G.K. Przybytniak, Radiat. Phys. Chem. 53 (1998) 491.
[2] M. Spotheim-Maurizot, F. Garnier, R. Sabattier, M. Charlier, Int. J. Radiat. Biol. 62 (1992) 659.
[3] B. Weiland, J. Huettermann, Int. J. Radiat. Biol. 74 (1998) 34.
[4] J.R. Milligan, J.A. Aguilera, J.F. Ward, Radiat. Res. 133 (1993) 151.
[5] M.T. Milano, W.A. Bernhard, Radiat. Res. 151 (1999) 39.
[6] I. Szumiel, M. Kapiszewska, M. Kruszewski, I. Iwanenko, C.S. Lange, Radiat. Environ. Biophys. 34 (1995) 113.
[7] R. Meneghini, Free Rad. Biol. Med. 23 (1997) 783.
[8] M.C.R. Symons, J.M.C. Gutteridge, Free Radicals and Iron: Chemistry, Biology and Medicine. Oxford University Press, Oxford, 1998 and references cited therein.
[9] J.D. Rush, W.H.J. Koppenol, Inorg. Biochem. 29 (1987) 199.
[10] D.Y. Yegorow, A.V. Kozlow, O.A. Azizova, Y.A. Vladimirow, Free Radic. Biol. Med. 15 (1993) 565.
[11] D.O. Lambeth, G.R. Ericson, M.A. Yorek, P.D. Ray, Biochim. Biophys. Acta 719 (1982) 501.
[12] S. Seibig, R. van Eldik, Inorg. Chem. 36 (1997) 4115.
[13] B. Halliwell, J.M.C. Gutteridge, FEBS Lett. 128 (1981) 347.
[14] J. Mirkowski, Program do analizy zdjec elektroforezy DNAnalyser, Raport IChTJ, B 4/98 (in Polish).
[15] M. Spotheim-Maurizot, M. Charlier, R. Sabattier, in: E.R.Balaban (Ed.), Frontiers in Radiation Biology, VCH, 1990, p. 493.
[16] T. Ito, S.C. Baker, C.D. Stickley, J.G. Peak, M.J. Peak, Int. J. Radiat. Biol. 63 (1993) 289.

[17] J. Dahm-Daphi, C. Sab, W. Alberti, Int. J. Radiat. 76 (2000) 67.
[18] I.L. Yurkova, H.-P. Schuchmann, C. von Sonntag, J. Chem. Soc., Perkin Trans. 2 (1999) 2049.
[19] B. Halliwell, J.M.C. Gutteridge, Free Radicals in Biology and Medicine, Oxford University Press, Oxford, 1989.
[20] B. Hoebel, C. von Sonntag, J. Chem. Soc., Perkin Trans. 2 (1998) 509.
[21] T.V. Rao, B. Sain, P.S. Murthy, T.S.R.P. Rao, A.K. Jain, G.C. Joshi, J. Chem. Res. (S) (1997) 300.
[22] W.H. Koppenol, J.F. Liebman, J. Phys. Chem. 88 (1984) 99.
[23] S. Goldstein, D. Meyerstein, G. Czapski, Free Rad. Biol. Med. 15 (1993) 435.
[24] V. Balzani, V. Carrasiti (Eds.), Photochemistry of Coordination Compounds, Academic Press, London, 1970, p. 172.
[25] J.M.C. Gutteridge, Biochem. J. 224 (1984) 697.