Radiat Environ Biophys (1986) 25:159–173

Radiation and Environmental Biophysics
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Radiation damage to φX174 DNA and biological effects

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Received June 15, 1986 / Accepted June 27, 1986

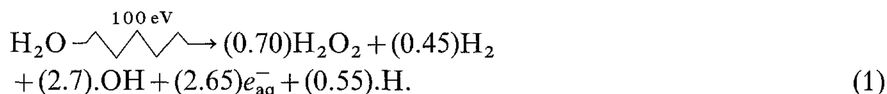
Summary. Dilute aqueous solutions of biologically active DNA can serve as a simplified model system of the cell. As a biological endpoint the survival of the DNA (after transfection to E. coli spheroplasts) is used. Damage in the DNA, irradiated in water with gamma rays, can be ascribed to reactions with primary waterradicals. By introducing additives in such solutions, which will scavenge the primary waterradicals, competition between a scavenger and DNA for such radicals can be studied. Comparison of different additives makes it possible to decide whether a compound behaves like a simple scavenger, radiosensitizer or like a radioprotector. In this context work has been done with the electron-affinic radiosensitizers metronidazole, misonidazole and nifuroxime. We have found that these wellknown cellular sensitizers do not enhance the inactivation of biologically active DNA. They act as simple competitive scavenger for waterradicals. However, if besides a sensitizer a trace of a metalloporphyrin containing compound (e.g. cyt. c) is present during irradiation an enhanced DNA inactivation, which can be interpreted as sensitization, is observed. Without sensitizer metalloporphyrins induce an enhanced protection of DNA.

Apart from these effects the consequences of both chemical-(sulphydryl) and enzymatic-(excision; recombination) repair has been studied. It has been found that sulphydryl compounds are able to react with DNA radicals, modifying the radiation damage in such a way that e.g. breaks are prevented. Further in double-stranded DNA a considerable amount of OH and also H radical damage appeared to be reparable by the excision-repair mechanism. However, post-replication repair had only very small or no effect on the amount of damage.

Introduction

It has been shown that radiation induced modifications of DNA are a major cause of cell killing and cell malfunctioning after irradiation. If we

want to investigate the mechanisms by which radiation affects the integrity of the DNA molecule at the molecular level, we have to reduce the relatively large number of parameters which are involved in in vivo systems. In this respect diluted aqueous solutions of DNA are suitable. In these solutions most of the radiation energy will be absorbed by the water. The radiolysis of neutral water by γ-rays can be summarized by means of the following symbolical expression:



$$H_2O \xrightarrow{100\,eV} (0.70)H_2O_2 + (0.45)H_2 + (2.7).OH + (2.65)e^-_{aq} + (0.55).H. \quad (1)$$

The figures between the parentheses represent the yields of the products, i.e. the number of molecules or radicals produced per 100 eV of absorbed energy. The number and/or yields of these products varies with the irradiation conditions as will be shown in the next section.

The purpose of the present paper is to discuss the reactions of the water-radiolysis products with DNA, how compounds such as radiosensitizers or radioprotectors are inferring and to assess the extent to which the biological functioning of DNA is affected by such reactions. These aspects can be studied by means of DNA isolated from the bacteriophage ϕX174. This circulary closed DNA can either be used in its single- or its double-stranded form, both DNA forms being biologically active when transfected to E. coli spheroplasts. This makes it also possible to study the influence of enzymatic repair on radiation induced DNA damage.

Comparison of the biological activity of DNA after irradiation with respect to that of unirradiated DNA gives a measure for the number of lethal events brought about by radiation. After steady state radiolysis of aqueous solutions of ϕX174 DNA exponential survival curves are obtained, indicating that lethality is brought about by a single-hit. Such an exponential survival curve is characterized by a D_{37} value, the dose at which 37% of the DNA molecules is still active and at the same time the dose needed to produce on the average one lethal event per DNA molecule (Poisson statistics).

$$N(D)/N_0 = \exp(-D/D_{37}). \quad (2)$$

In other words the D_{37} equals to the number of DNA molecules divided by the number of lethal events per unit dose and therefore is proportional to the concentration N_0 of DNA molecules divided by the yield G_b for the number of lethal events per 100 eV,

$$D_{37} = 100\ N_0/rG_b \quad (3)$$

in which r is the absorbed energy (in eV) per unit mass for one unit of absorbed dose ($r = 6.25 \times 10^{15}\ g^{-1}\,Gy^{-1}$).

Although it will be clear from the presented paper that diluted aqueous solutions of ϕX174 DNA is a suitable system to study in vitro biological effects of radiation, it should be stressed, however, that statements about relative effectiveness of different radicals or efficiencies of radical reactions

pertaining to the ϕX174 DNA system may not always apply to other systems.

Inactivation of DNA by water radicals

Of the irradiation products in water, the molecular products H_2, H_2O_2 do not give a significant contribution to the inactivation of DNA in pure solution, although a slow reaction is possible, particulary in the presence of contaminating metal ion impurities that may exert a catalytic reaction (see Blok and Loman 1973). That primary water radicals can be involved in DNA is illustrated by irradiation of 3.3 μg/ml single-stranded ϕX174 DNA under different gas conditions as performed by Lafleur et al. (1975). The yields (G-values) of the radicals present under the different conditions are given in Table 1. As can be seen from Fig. 1 irradiation under N_2O ($e^-_{aq} + N_2O + H_2O \rightarrow N_2 + OH^- + .OH$) makes the DNA about two times as sensitive towards radiation as under oxygen indicating the importance of the OH radical. Further it shows that in these diluted aqueous DNA solutions oxygen is protective by scavenging the reducing water radicals. Apparently the radical anion $O_2^{\overline{\cdot}}$ which is formed in these reactions with oxygen does not give a significant contribution to the inactivation of DNA in solution. Irradiation under nitrogen in sodium-chloride containing solutions reveals that the hydrated electron is by far not so effective as the .OH radical. To get a better idea about the effectiveness of H radical the yield of this radical is increased by irradiation in a phosphate buffer in which the hydrated electrons are converted into H radicals ($e^-_{aq} + H_2PO_4^- \rightarrow .H + HPO_4^{2-}$) and the yield is now comparable to that of the OH radical if irradiated under nitrogen. With respect to the results in the sodiumchloride solutions only the nitrogen curve is shifted significantly. Comparing the yields of the H and the OH radical (see Table 1) this shift in the nitrogen curve indicates that the H radical is equally as effective in DNA inactivation as the OH radical. So one should be careful in neglecting the possible contribution of H radicals to biological effects. Further the experiments show that if there is biological inactivation by the hydrated electron, it must be very small. In more sensitive experiments Nabben et al. (1982) eliminated .OH and .H. Under these conditions an inactivation was found that must be due to e^-_{aq}. They found that the biological effect of e^-_{aq} is about 10 times smaller than that of .OH.

Nabben et al. (1984) assessed the effectiveness of .OH, .H and e^-_{aq} in dilute aqueous solutions of double-stranded (RF)DNA. It appeared that the double-stranded form is less sensitive than the single-stranded form of ϕX174 DNA. Furthermore .OH and .H radicals are again about equally effective in inactivating. However, no significant biological effect of hydrated electrons could be detected for double-stranded DNA.

Inactivation efficiency and non-homogeneous kinetics

In the previous experiments the effectiveness of the different waterradicals in DNA inactivation was studied. This effectiveness can be expressed as

Table 1. G-values of primary water radicals under three different gas conditions; a) in a 10^{-2} mol dm^{-3} phosphate buffer, b) in a 2.10^{-2} mol dm^{-3} NaCl solution

Gas	a)			b)		
	G(OH)	G(H)	$G(e_{aq}^-)$	G(OH)	G(H)	$G(e_{aq}^-)$
O_2	2.7	–	–	2.7	–	–
N_2	2.7	3.25	–	2.7	0.55	2.7
N_2O	5.4	0.55	–	5.4	0.55	–

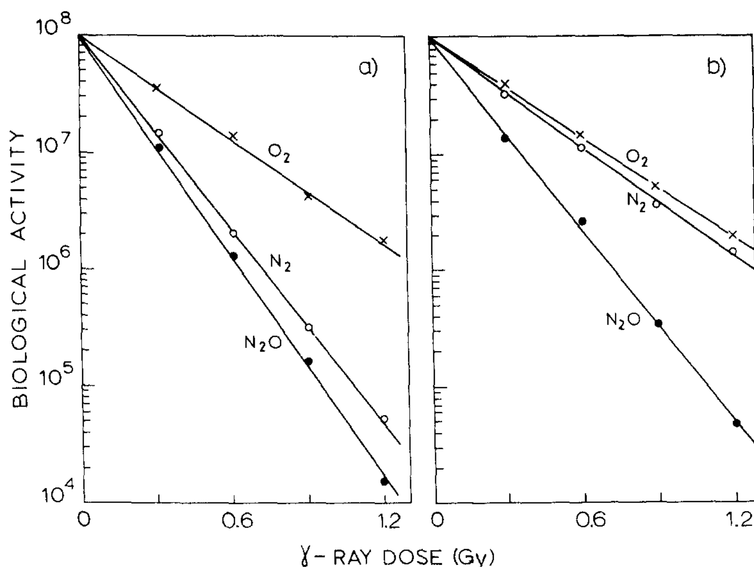


Fig. 1a and b. Survival curves for single-stranded ϕX174 DNA (10^{-5} mol dm^{-3} nucleotides), irradiated in a solution containing: **a** 10^{-2} mol dm^{-3} phosphate buffer pH 7.2; **b** 2.10^{-2} mol dm^{-3} NaCl pH ≈ 7, under three different gasconditions -●- N_2O; -o- N_2; -x- O_2

the efficiency ε of the reaction being lethal times the fraction f of radicals reacting with DNA

$$G_b/G = \varepsilon f = \text{effectiveness} \tag{4}$$

It would be interesting to know the efficiency for a particular radical. A simple way to determine this efficiency seems to perform a competition experiment for such a radical, so changing the factor f explicitly. This can be done by adding to the DNA solution compounds which protect the DNA against the reaction of water radicals by scavenging of these radicals. In Fig. 2 the result of such an addition (phenol) to oxygen-saturated solutions of DNA is presented. It is clear that this gives protection of the DNA, resulting in a higher radiation dose necessary for the same percentage of survival of the DNA. This protection depends on the concentration of the added scavenger and the rate of reaction with a particular waterradical, the so-called scavengercapacity ($K_s S$). If we assume that a scavenger is

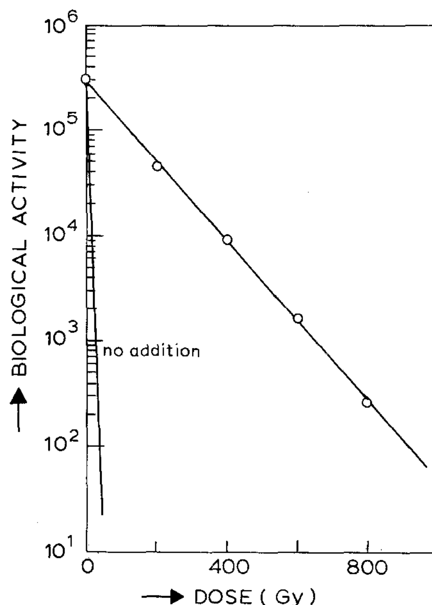


Fig. 2. Survival curves of single-stranded ϕX174 DNA irradiated in oxygen satured solutions containing 10^{-5} mol dm^{-3} (nucleotides) DNA, 10^{-1} mol dm^{-3} NaCl, 2.10^{-2} mol dm^{-3}, phosphate buffer (pH = 7) (—) no further additon; (-o-) with 5.10^{-3} mol dm^{-3} phenol [kS(1/s) = 7×10^7]

present at a concentration S and that it reacts with a radical R with a rate constant K_s we can write the biological inactivation yield as

$$G_b = \varepsilon G_R \, K_0 N_0 / (K_0 N_0 + k_s S). \qquad (5)$$

Here K_0 is the rate constant for reaction of a radical with DNA molecules and ε is the inactivation efficiency. Substitution of this expression in Eq. (3) gives

$$D_{37} = (100/r\varepsilon G_R)\,(N_0 + k_s S/K_0). \qquad (6)$$

By determining the value of D_{37} as a function of the scavengercapacity the inactivation efficiency ε can be assessed. One would expect a linear plot of the D_{37} value against the scavengercapacity of the solution. However, if we do such an experiment for the reaction of the OH radical in oxygen saturated solutions of single-stranded ϕX174 DNA and OH radical scavengers like formate, phenol and isopropanol, we do not obtain a straight line (Fig. 3).

The bending of the competition curve can not be explained by the presence of reactive secondary scavenger radicals because it is unlikely that all the different scavenger radicals are equally effective in DNA killing. The bending of the competition curve can be explained, however, if one visualizes the DNA molecule in solution as a sphere in which the nucleotide concentration is high.

At low scavenger concentrations the scavenging capacity of the solution inside the DNA sphere is much greater than outside this sphere. Therefore all radicals which arrive in this spherical region will react with the DNA molecule. So as a first approximation we might consider the DNA molecule to be a compact sphere (Van Rijn et al. 1985). The diffusion controlled

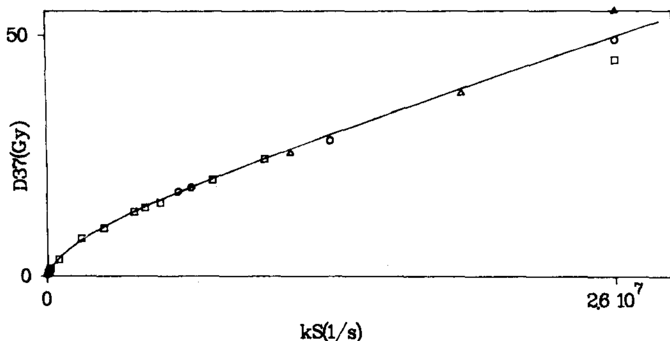


Fig. 3. Dependence of the D_{37}-value of single-stranded φX174 DNA on the scavenging capacity for OH radicals: (□) phenol; (0) formate and (△) isopropanol. The oxygen saturated solutions contained 10^{-5} mol dm^{-3} (nucleotides) DNA and 10^{-1} mol dm^{-3} NaCl (with or without 10^{-2} mol dm^{-3} phosphate buffer (pH≈7). The dose-rate used was 0.6 Gys^{-1}. The line is a fit to the experimental data according to the model described in the section: inactivation efficiency and non-homogeneous reaction kinetics

reaction rate constant for reaction of a radical with such a sphere is given by

$$k_s = 4\pi R_s D[1 + \alpha_2 R_s] \quad \text{with } \alpha_2 = (k_s S/D)^{\frac{1}{2}} \tag{7}$$

where R_s is the effective reaction radius of the DNA sphere en D is the diffusion constant of the radical.

At high scavenger concentrations only the radicals formed inside the DNA sphere have a change to react with the DNA molecule with a reaction rate constant which effectively is a reaction rate constant for reaction with a cylinder coiled in a sphere of radius R_s. Although this reaction rate constant also depends on the scavenging capacity (Verberne 1981), this dependence is still insignificant in our experiments and we can derive the following scavenger independent reaction rate constant

$$k_c = 2\pi LD \frac{1}{-\ln(\varrho) - (\varrho^4/4 - \varrho^2 + 3/4)} \quad \text{with } \varrho = R_0/R_c \tag{8}$$

where R_0 is the reaction radius of the cylinder, R_c is half the average distance between cylindrical segments of DNA within the sphere R_s and L is the contour length of the DNA cylinder. R_c is found by putting the cylindrical volume available for the cylinder equal to the volume of the sphere with radius R_s: $\pi R_c^2 L = 4\pi R_s^3/3$.

k_s will be smaller than k_c therefore k_0 increases with the scavenger concentration. This decreases the dependence of the D_{37}-value on the scavenger concentration resulting in a bending of the competition curve.

For the fitting of the experimental points we have also taken into account the fraction of radicals formed inside and outside the DNA sphere and the competition within the sphere between the scavenger and the DNA cylinder, resulting in the following reaction rate constant (Verberne 1981)

$$\frac{k_0}{k_c} = [1 + \frac{3}{(\alpha_1 R_s)^2} F] \, [1 - (\alpha_2/\alpha_1)^2] \tag{9}$$

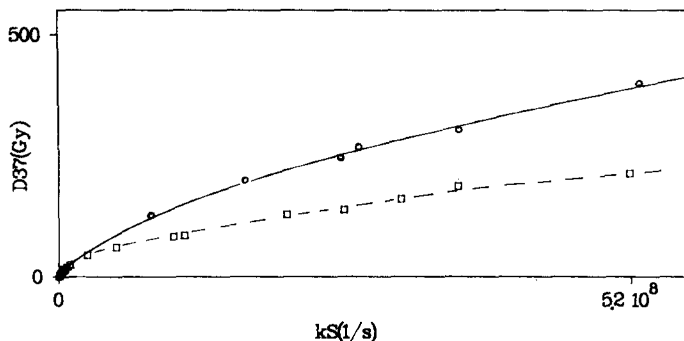


Fig. 4. Dependence of the D_{37} value of single-stranded ϕX174 DNA on the scavenging capacity of OH radicals: (□) t-butanol; (0) phenol. The oxygen saturated solutions contained 10^{-5} mol dm^{-3} (nucleotides) DNA, 10^{-1} mol dm^{-3} NaCl, 10^{-2} mol dm^{-3} phosphate buffer (pH 7.2) and were irradiated using a dose-rate of 0.6 Gys^{-1}. The full line drawn in this figure is a fit according to the model developed by Verberne et al. (1986)

where

$$F = \frac{1-y}{y+(1-y)/(1+(\alpha_2 R_s))} \quad \text{with} \quad y = \text{tgh}(\alpha_1 R_s)/(\alpha_1 R_s)$$

where $\alpha_1 = [(k_c C + k_S S)/D]^{\frac{1}{2}}$ (C is the effective concentration of the cylinder within the sphere R_s) and α_2 is defined in Eq. [7].

From the fitting of the experimental data in Figure 3 it follows that the radius of the equivalent DNA sphere is about 80 nm, which is comparable to the radius of gyration of single-stranded ϕX174 DNA under these conditions, that the inactivation efficiency of the OH radical is about 20%, and that $k_c = 2 \times 10^9$ mol^{-1} dm^3 s^{-1} per nucleotide. Using expression [8] for k_c this last value corresponds to a reaction radius of the DNA cylinder of about 0.5 nm, which does not seem to be unrealistic. Similar experiments have also been done for the hydrated electron reaction with single-stranded ϕX174 DNA resulting in an inactivation efficiency of about 8% (Nabben et al. 1982).

At still higher scavenger capacities, this type of experiments were extended up to twenty times higher scavenging capacities (see Fig. 4), one has to take into account that OH radicals are already scavenged in the spur resulting in an affective increased yield of the water radicals and possibly even a contribution of the direct effect. However, fitting the experimental data (Verberne et al. 1986) yields about the same values for the parameters R_c, K_c and ε as found in the lower scavenging capacity region (see above).

Inactivation of DNA by secondary radicals

An example of the damaging reaction by secondary radicals can be seen in Fig. 4. Here the common OH radical scavenger t-butanol is used as a scavenger. The curve for t-butanol deviates, especially at higher concentrations, from the general competition curve as obtained with the other scavengers, implying more lethal damage than can be expected, although the DNA

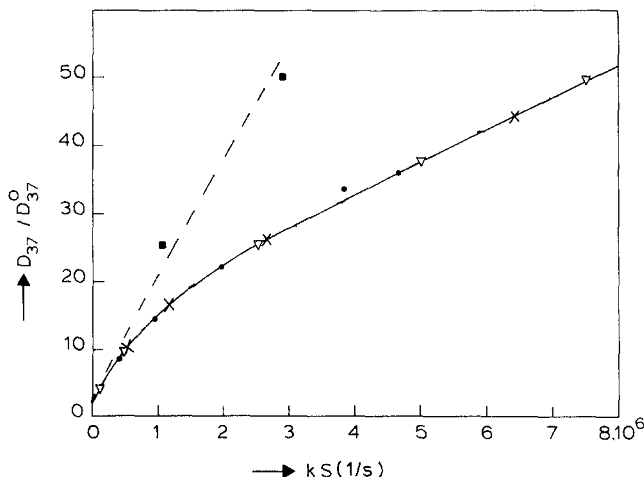


Fig. 5. Competition curve for the biological inactivation: the normalized D_{37} (D_{37}/D_{37}^0) versus scavenger capacity [ks(1/s)]. 10^{-5} mol dm^{-3} (nucleotides) single-stranded ϕX174 DNA irradiated in 10^{-1} mol dm^{-3} NaCl (pH≈7): (■) cysteamine under N_2O, (●) phenol under N_2O, (▿) phenol under O_2, (x) formate under O_2. The full line is again the fit according to the model developed in the section: inactivation efficiency and non-homogeneous reaction kinetics

is still protected against the reaction of the OH radical. This indicates that the radicals formed out of t-butanol through the reaction with .OH are not as effective in inactivating DNA as OH radicals. That indeed secondary radicals are involved could be demonstrated by a dose-rate effect on this extra damage. Lowering the dose-rate increased the amount of damage. With the scavengers of the general competition curve no such effect could be detected. Also with other secondary radicals such as radicals from amino-acids inactivation of ϕX174 DNA has been found (de Jong et al. 1972).

Chemical repair

A general competition curve can also be used to decide whether a scavenging compound gives an *extra* protection to the DNA. A nice example of such an extra protection is given by sulphydryl compounds such as cysteamine. From Fig. 5 it is quite clear that cysteamine offers more protection against inactivation of single-stranded ϕX174 DNA than can be expected on basis of just simple scavenging of waterradicals. In this figure the DNA survival is expressed as D_{37}/D_{37}^0 to make a comparison for the different irradiation conditions possible, i.e. taking into account the differences in radical yields. D_{37}^0 is the 37% survival dose at zero scavenger concentration. Perhaps part of the initially damaged sites in the DNA molecules are repaired by cysteamine, possibly by H donation as has been proposed by Howard-Flanders (1960) and Hutchinson (1961). As can be deduced from the work of Lafleur et al. (1980) at least part of this chemical repair is due to prevention of breaks, a damage which is lethal in single-stranded ϕX174 DNA. Assuming sugar damage as prerequisite for the production of breaks, this prevention by cysteamine can be explained by a repair reaction through hydrogen atom

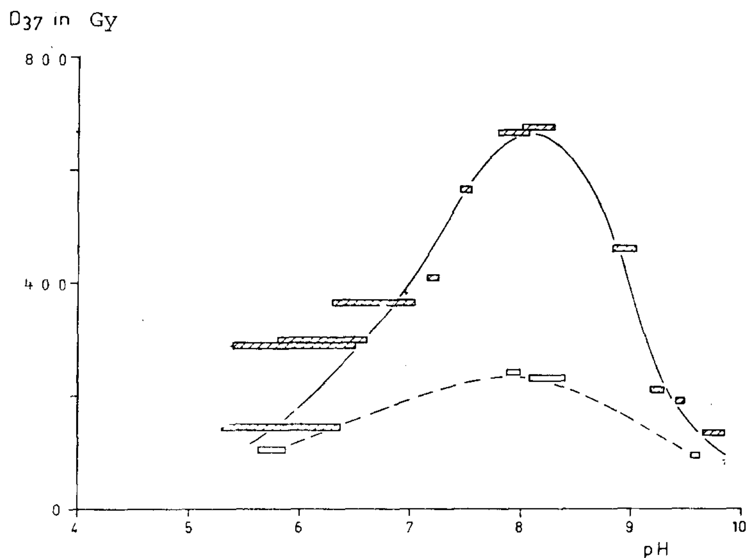


Fig. 6. The D_{37} as function of pH. Single-stranded ϕX174 DNA (1.2×10^{-5} mol dm^{-3} nucleotides) was irradiated in the presence of 5.10^{-3} mol dm^{-3} cysteamine. ▨, in nitrogen; □, in oxygen. The uncertainties represent the pH before irradiation and after the highest dose. During irradiation the pH shifted to higher values, particularly at low pH

transfer to the damaged sugar moieties in DNA, e.g. to the 4′ and 1′ carbon radical, which could otherwise have led to breaks (Dizdaroglu et al. 1977a, b; von Sonntag 1984). Further it is found (see Fig. 6) that the observed extra protection of the DNA by cysteamine depends largely on the pH of the solution (Blok 1967; Lafleur et al. 1980) and the presence of oxygen (Blok 1967). The pH dependence of the protection of DNA by cysteamine or cysteine is different from that found by Adams et al. (1968) for the repair reaction of cysteamine with methanol radicals. This might be due to pH dependent properties of DNA radicals. Finally the results support the idea that oxygen and sulphydryl compounds are competing for the same damaged sites in DNA leading to fixation or repair of the damage.

Enzymatic repair

Apart from the chemical repair reactions as shown above, damaged DNA can also be subject of the action of the various DNA repair enzymes. In order to investigate whether damage due to water radicals is repaired by one of these enzymes and, whether any difference exists in the type of damage introduced by e.g. H or OH radicals, experiments were carried out in which the biological activity of double-stranded (RF) ϕX174 DNA was determined with spheroplasts prepared from strains which are deficient in one of the genes of the repair systems. An example is given in Fig. 7. Here we see the effect of the survival of DNA on mutant compared to the wild-type spheroplasts. This mutant is deficient in part of the excision repair system, particularly the gene which codes for the uvr A protein.

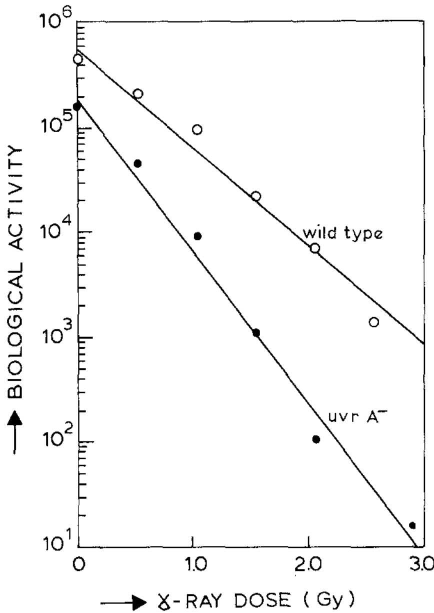


Fig. 7. Survival curves for double-stranded ϕX174 (RF) DNA (6×10^{-6} mol dm^{-3} nucleotides) dissolved in 10^{-1} mol dm^{-3} NaCl + 10^{-2} mol dm^{-3} phosphate buffer (pH 7.2) irradiated under nitrogen. After the irradiation experiment the biological activity was determined on wild-type spheroplasts (E. coli AB 1157) –o–, and on uvr A$^-$ spheroplasts (E. coli AB 1886) –•–

Table 2. Relative increase of radiosensitivity of ϕX174 RF DNA, measured after transfection to different repair-deficient mutant spheroplasts and compared with the wild-type results. The data presented are derived from Nabben et al. (1984)

Mutant sferoplast	D_{37} [wild-type] / D_{37} [mutant]		
	anoxia		oxygen
	100% .H	100% .OH	100% .OH
uvrA$^-$	2.1 ± 0.2	1.1 ± 0.1	1.0 ± 0.1
recA$^-$	1.4 ± 0.3	0.9 ± 0.1	
uvrC$^-$	1.1 ± 0.2	1.6 ± 0.1	1.0 ± 0.1

An increase in the inactivating damage as measured on the uvr A$^-$ spheroplasts is observed. Apparently part of the damage can be recognized by the uvr A protein and becomes repaired in the wild-type. In Table 2 results are summarized of experiments in which ϕX174 (RF) DNA was irradiated unter various conditions in order to determine repair of damage introduced by H or OH radicals (Nabben et al. 1984). The mutants used were deficient in certain parts of excision repair mechanism, these deficiencies are indicated by uvr A$^-$ or uvr C$^-$, or in a post-replication repair mechanism rec A$^-$. From the experiments with oxygen it is clear that under those circumstances the excision repair mechanism does not effect the damage, caused by OH radicals. Under anoxic conditions (N_2, N_2O) it can be seen that several values in the table differ significantly from 1 revealing that repair is involved. The results indicate that under these anoxic conditions part of the radiation damage due to OH radicals is repairable by an uvr C gene dependent mechanism, whereas this repair pathway does not play an important role in case of the H radical. The reversed situation applies to the uvr A gene dependent

repair, which only effects the H radical damage. Furthermore the post-replication repair mechanism (rec A) has only a very small effect on the amount of inactivation by .H and none at all for .OH. As far as hydrated electrons are concerned we did not find a significant inactivation of the ϕX174 (RF) DNA, whether the biological activity was determined by use of wild-type or different mutant spheroplasts. Although it is clear that the experiments with repair deficient mutants show differences between damage caused by the various water radicals, relations between repair pathways on one hand and various types of damage on the other remain rather speculative and they will not be presented here. For a specific discussion the reader is referred to the paper by Nabben et al. (1984).

Radiosensitization

It would also be very interesting to see whether compounds which have been shown to behave like radiosensitizers in cellular systems also behave as such in a model system like ϕX174 DNA in aqueous solution. This could provide more knowledge about the mechanisms of radiosensitization. For that purpose competition experiments have been carried out with the electron-affinic radiosensitizers misonidazole, paranitroacetophenone (PNAP) and nifuroxime (Lafleur et al. 1982). These radiosensitizers are all good scavengers of the waterradicals like the OH radical. Therefore they will, besides possible sensitization, protect the DNA. As can be seen in Fig. 8 the data obtained with the radiosensitizers fall on the same general competition curve. It is clear that under these experimental conditions they act like ordinary scavengers for water radicals and not as radiosensitizers with respect to the inactivation of single-stranded DNA. The same conclusion can be drawn from experiments with double-stranded DNA.

Further attention is paid to the different types of radiation damage in single-stranded DNA (Lafleur et al. 1982). The most striking observation is an increase in acute breaks accompanied by a decrease in latent breaks, the latter can be revealed by an alkali treatment, in the presence of misonidazole and nifuroxime under anoxic conditions. However, this does not cause extra inactivation because both types of breaks are lethal and the total contribution of breaks (acute + latent) remains unchanged. This shift in type of breaks in possibly due to electron-transfer from a damaged deoxyribose to the sensitizer as suggested in the literature (Michaels et al. 1976). The results presented so far suggest that something more is needed for a sensitizer to sensitize and which must be a natural constitutent of living cells. A more natural environment from a radiobiological point of view can be achieved by dissolving the DNA in an extract of bacteria or cells. Indeed sensitization can then be demonstrated, for instance for double-stranded DNA by oxygen and the radiosensitizers TAN and PNAP (van Hemmen et al. 1974a, b) and for single-stranded DNA by misonidazole (Lafleur et al. 1982a). The bulk of organic material in such an extract was shown to behave like a simple radical scavenger (Lafleur et al. 1982a). Therefore it can be expected that the extract as such is not required for the activation of sensitizers, but that only the "activator" has to be present. As possible

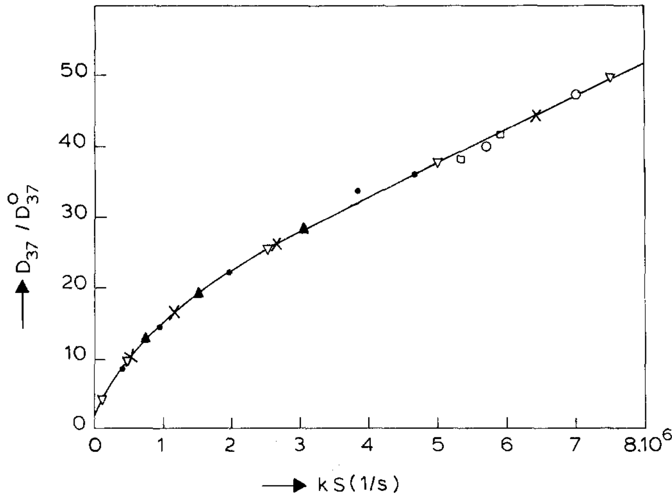


Fig. 8. Competition curve for the biological inactivation. 10^{-5} mol dm^{-3} (nucleotides) single-stranded ϕX174 DNA, irradiated in 10^{-1} mol dm^{-3} NaCl, pH≈7; (x) misonidazole under N_2O (O) under N_2, (□) under O_2; (●) nifuroxime under N_2O; (▲) paranitroacetophenone; (▽) phenol under O_2. The line drawn in this figure is the same as the full line in Fig. 5

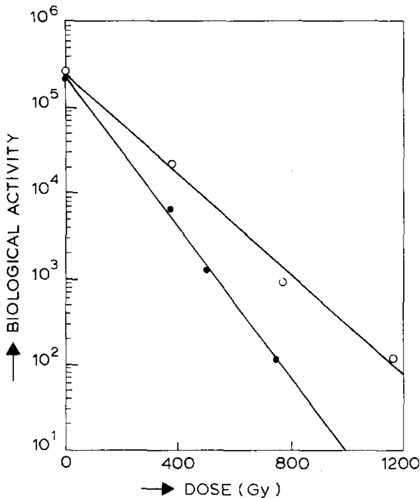


Fig. 9. Survival curves of single-stranded ϕX174 DNA irradiated in nitrogen saturated solutions containing 10^{-5} mol dm^{-3} (nucleotides DNA), 10^{-1} mol dm^{-3} NaCl. 10^{-2} mol dm^{-3} phosphate buffer (pH 7), 10^{-2} mol dm^{-3} misonidazole –o– without cytochrome c, –●– with 10^{-6} mol dm^{-3} cytochrome c

candidates already suggested in the literature (van Hemmen et al. 1978) the metalloporphyrins were chosen. Addition of e.g. a small amount of cytochrome c (or hemin) gives rise to an increased inactivation of single-stranded ϕX174 DNA irradiated together with a sensitizer as can be seen in a typical example with misonidazole (Fig. 9) yielding an enhancement ratio of about 1.7. The enhancement ratio is defined by the ratio of the slopes (Lafleur et al. 1982b). For scavengers which have been shown not to sensitize like phenol etc. no sensitizing effect of the addition of a metallo-prophyrin has been found but in contrast an extra protecting effect (Lafleur

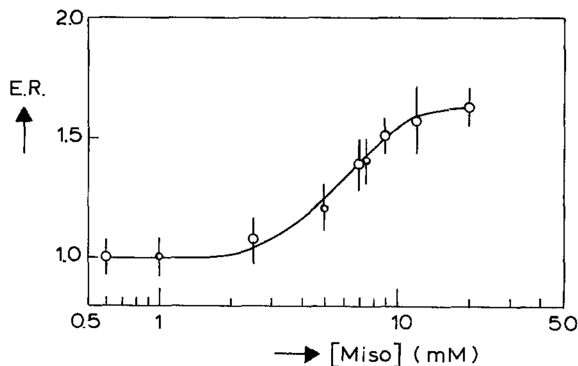


Fig. 10. The enhancement ratio (ER) as a function of concentration of misonidazole. Single-stranded ϕX174 DNA 10^{-5} mol dm^{-3} nucleotides) was irradiated under nitrogen dissolved in 10^{-1} mol dm^{-3} NaCl + 10^{-3} mol dm^{-3} borate buffer (pH 6.9). Error bars represent standard errors

et al. 1983, 1984). This protection is larger than can be expected on basis of the additional scavengercapacity of the metalloporphyrin. Perhaps one or more of the initial damaged sites in the DNA are chemically repaired. At least there is a resemblance with the action of cysteamine, because the enhanced protection of the DNA shows a pH dependence which is similar to that found with cysteamine (see Fig. 6). In this context it is possibly of interest that porphyrins tend to accumulate in tumours when given to animals and man (Tsutsui et al. 1975) and that anoxic human cells supplied with haematoporphyrin (but not with radiosensitizer) are protected against inactivation by X-rays (Moan and Pettersen 1981). The main conclusion sofar must be that the simultaneous presence of a sensitizer and a metalloporphyrin is necessary and sufficient to evoke sensitization. If one of these compounds is absent no sensitization is found: The sensitizers behave like ordinary radical scavengers and the metalloporphyrins offer even an extra protection to the DNA.

The extra inactivation as found in the presence of a radiosensitizer and cytochrome c depends on the concentration of the radiosensitizer (Lafleur et al. 1984). This dependence of which an example is given in Fig. 10 is characterized by a clear S shaped curve and resembles the curves obtained with bacteria and mammalian cells (Fielden et al. 1978; Moore et al. 1976).

Further it has been established that in this relatively simple system of single-stranded ϕX174 DNA/sensitizer/cytochrome c the sequence of sensitization efficiencies is the same as for cellular sensitization (metronidazole < misonidazole < nifuroxime). However, in a quantitative sense, the dependence of this efficiency on the one-electron redox potential is less pronounced for DNA.

We also investigated the nature of the extra inactivating damage in the DNA (Lafleur et al. 1985). After irradiation of single-stranded ϕX174 DNA in the presence of misonidazole and cytochrome c (enhancement ratios of 1.7–1.9), the samples were subjected to a mild alkaline treatment. This treatment results in a considerable increase of survival, almost back to the situation without cytochrome c, indicating that an inactivating adduct is released thereby restoring the activity of the DNA molecule. Without misonidazole or cytochrome c no such an effect could be observed. In summary

it can be said that very probably radiosensitization by misonidazole/cytochrome c of single-stranded ϕX174 DNA in solution is due to prevention of the extra protection of the DNA offered by the metalloporphyrin and increase of the radiosensitivity of the DNA by inflicting extra damage into the DNA. This extra damage is probably due to the radiation-induced formation of an adduct between sensitizer (products) and DNA.

Conclusion

We have shown some aspects of the radiation chemistry of ϕX174 DNA in diluted aqueous solutions and how it can provide some interesting answers and suggestions. It is amazing how much of the radiation induced damage in DNA is either irrelevant or can be repaired even in single-stranded DNA. Further it will be clear that many questions are still open and even raised by the experiments presented and further research is needed to elucidate these questions.

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