Effects of Ionising Radiation on Deoxyribonucleic Acid. Part 3.† The Effect of Iodoacetamide

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Exposure of frozen aqueous solutions of calf thymus DNA to ^{60}Co γ-rays at 77 K gave equal yields of electron-gain centres localised on thymine (T^-) and electron-loss centres localised on guanine (G^+) as judged by e.s.r. spectroscopy. In the presence of iodoacetamide (ICH_2CONH_2) electron capture by the additive to form $^{\bullet}CH_2CONH_2$ and I^- occurred in competition with electron capture by thymine. The yield of G^+ remained unaffected as estimated from e.s.r. spectra using computer simulation. On annealing, the e.s.r. features for $^{\bullet}CH_2CONH_2$ were lost at *ca.* 190 K, with the simultaneous appearance of signals that were assigned to DNA radicals. Analysis for chemical damage using plasmid DNA (pBR 322), irradiated under similar conditions, revealed an increase in the number of single strand-breaks and approximately no change in the number of double strand-breaks arising from the presence of iodoacetamide. Various interpretations of the e.s.r. spectra of the secondary DNA radicals are offered, the most probable being that at least one of these species is formed by hydrogen-atom abstraction from the sugar moiety. We suggest that the increase in single strand-breaks occurs because, on warming, the trapped $^{\bullet}CH_2CONH_2$ radicals react with DNA to give sugar radicals, at least one of which leads to strand breaks. The observation that the sugar radicals are lost at temperatures below those at which the DNA radicals detected by e.s.r. normally decay, offers an explanation of why sugar radical intermediates have not been detected in our previous studies.

At the cellular level, iodoacetamide (IA) has for many years been known to be a powerful radiosensitising agent.[1,2] Explanations offered for this *in vivo* sensitising effect include inhibition of protein,[3-5] RNA,[5] and DNA[4,5] synthesis as well as inhibition of slow[5,6] and fast[7] DNA repair processes. As part of our systematic study of the effects of additives on the course of radiation damage to DNA and other biopolymers, we have undertaken a detailed e.s.r. and strand-break study of the role of iodoacetamide at the molecular level.

Experimental

Materials.—Plasmid DNA (pBR 322) was isolated according to a modified procedure of Birnboim and Doly.[8] Typically, pBR 322 DNA preparations contained *ca.* 95% of the superhelical Form I DNA. Tris buffer, EDTA, ethidium bromide, and calf thymus DNA (for e.s.r. studies) were obtained from the Sigma Chemical Company. Agarose-ME was obtained from the Miles Laboratory. [$^2H_3$]Methyl alcohol (99.5 atom %), D_2O, and iodoacetamide were purchased from the Aldrich Chemical Company. [$^2H_3$]Iodoacetamide was obtained from Lancaster Synthesis Ltd.

γ-Irradiation and Assays for DNA Breaks.—The assay used has been described in our previous publications.[9,10] Variations were introduced in order to optimise the separation of the linear Form III DNA from the relaxed Form II DNA. A solution of iodoacetamide (10 μl) was added to the plasmid DNA solution (90 μl; 80 μg ml^{-1}) in Tris HCl buffer (10mM): pH 7.6) containing EDTA (1mM) to give the appropriate final iodoacetamide concentration. Samples with and without iodoacetamide additive were γ-irradiated in a Vickrad ^{60}Co γ-ray source under the appropriate conditions. Oxygenated and deoxygenated samples were prepared by gas purging with oxygen or oxygen-free nitrogen for 60 min then sealed and irradiated. Following irradiation a dye–EDTA mixture (6 μl; 56% glycerol v/v; 50mM–EDTA; 0.05% Bromophenol Blue w/v) was added and portions removed for analysis by agarose gel electrophoresis.

Gel Electrophoresis.—Samples of irradiated DNA (0.7—1 μg) were layered onto agarose slab gels (1.3%) and electrophoresed in a horizontal gel apparatus at 40 V for *ca.* 16 h at room temperature using Tris buffer (90mM, pH 8.3) containing boric acid (90mM) and EDTA (2.5mM). After electrophoresis the gels were stained with ethidium bromide (2.5 μg ml^{-1}) in electrophoresis buffer for at least 15 min and the excess removed by washing. The stained gels were then excited with a trans-illuminator and photographed with a Polaroid MP-4 Land Camera using a red filter (Kodak Wratten filter No. 9) and Polaroid type 55 film. The negative films of gels were used for densitometric scanning.

Quantitation of Single and Double Strand-breaks.[11]—The negative films of the ethidium bromide-stained gels were scanned using a u.v.-visible spectrophotometer with gel scanning attachment (SP8-100 Pye-Unicam). The superhelical Form I of the plasmid takes up less ethidium bromide than the other two Forms. We have used a staining efficiency of 80% (as demonstrated for pBR 322[12]) to normalise our data. No further uptake of ethidium bromide was observed on prolonged incubations. All samples were analysed in duplicate and average values reported in the Results section.

γ-Irradiation and E.s.r. Measurements.—Frozen samples were prepared by cooling in liquid nitrogen a Pyrex tube containing a solution of DNA (50 mg ml^{-1}; Type I sodium salt DNA; Sigma) with and without iodoacetamide (20mM-additive unless otherwise stated). Samples were irradiated as described above, and the e.s.r. spectra recorded (*X*-band Varian E-109

† Part 2, ref. 10.

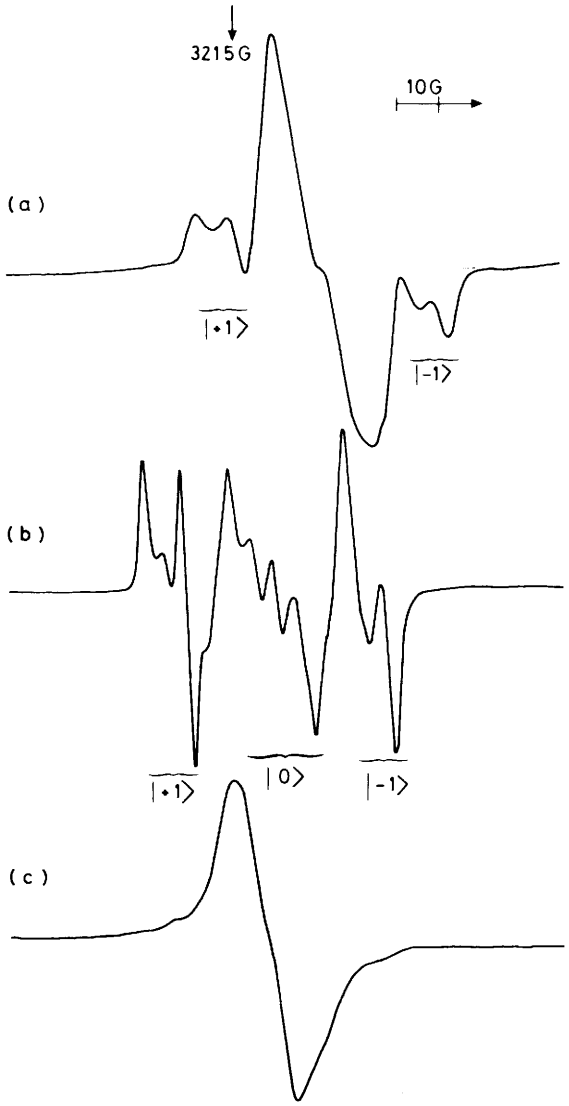


Figure 1. First-derivative *X*-band e.s.r. spectra for iodoacetamide radicals formed by exposure of various systems containing 20mM-iodoacetamide to γ-rays at 77 K: (a) aqueous DNA after annealing to *ca.* 130 K to remove 'OH radicals formed in ice crystals; (b) D_2O + CD_3OD after annealing to remove solvent radicals; and (c) as in (a) but using ICD_2COND_2. In (a) and (c) the central component is made up of the $M_I = 0$ feature together with features for DNA radicals

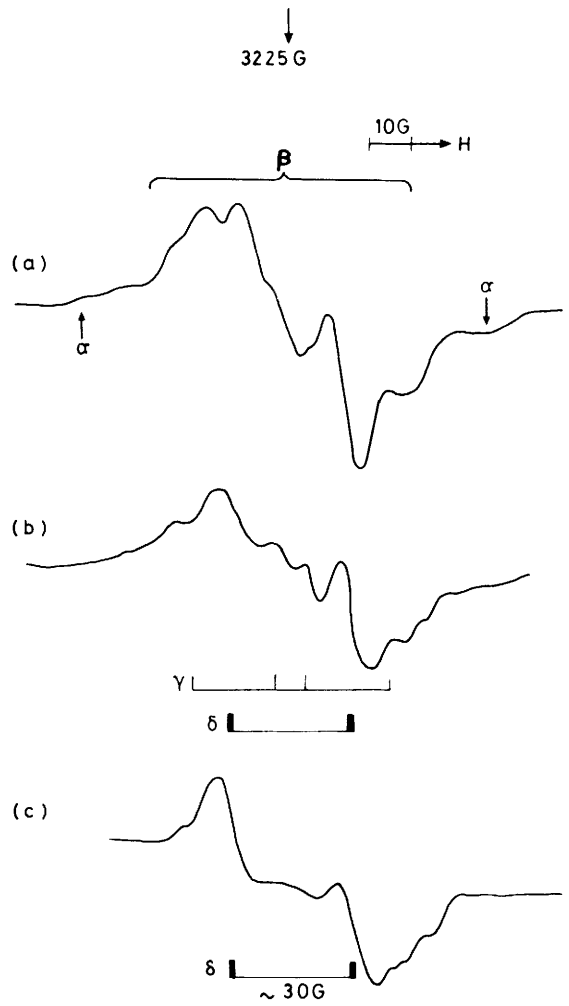


Figure 2. First-derivative *X*-band e.s.r. spectra for iodoacetamide plus deoxygenated DNA systems after annealing to remove features due to $^{\cdot}CH_2CONH_2$ radicals to reveal features referred to in the text as species α, β, γ, and δ. (a) One of a series of spectra obtained in the temperature range 220—240 K. Outer features (α) are probably not due to the same species as the central 'quartet' referred to as species β (b) and (c). After further annealing, 240—250 K, showing the loss of β and the growth of other features, γ and δ, as discussed in the text

spectrometer, 100 KHz field modulation). Spectra were stored and used for computer simulations and subtractions as outlined previously. *G* Values (numbers of radicals or strand breaks per 100 eV) were estimated by comparison with the double integral value taken from a spectrum of di-t-butylnitroxyl of known spin concentration.

Results

E.s.r. Studies.—Irradiation of frozen aqueous solutions of DNA at 77 K gave rise to an e.s.r. spectrum that comprised features for trapped OH' in ice crystallites together with features from DNA radicals. The latter have previously been assigned to T^- and G^+.[9,13] In oxygenated systems features for O_2^- and $HO_2^{\cdot}$ radicals were also detected. The hydroxyl radicals, being phase separated from the DNA, were lost on annealing to *ca.* 130 K with no significant effect on the DNA radicals.

In the presence of iodoacetamide at a drug:base ratio of *ca.* 1:6 additional features assigned to $^{\cdot}CH_2CONH_2$ radicals were also detected. These features were well defined after annealing to remove the 'OH radical spectrum (Figure 1a). They comprise an asymmetric triplet due to hyperfine coupling to the two α protons. This was confirmed by generating authentic $^{\cdot}CH_2CONH_2$ by irradiating iodoacetamide in a CD_3OD–D_2O mixture where electron capture is the dominant process (Figure 1b). Assignment of the outer, $M_I = \pm 1$, features to $^{\cdot}CH_2CONH_2$ radicals was confirmed by study of the deuteriated iodoacetamide. As expected, these features were no longer detectable, those for $^{\cdot}CD_2CONH_2$ radicals being part of the central component (Figure 1c).

It seems that iodoacetamide undergoes dissociative electron capture (1), in competition with electron capture at thymine.

$$ICH_2CONH_2 + e^- \longrightarrow {}^{\cdot}CH_2CONH_2 + I^- \quad (1)$$

Computer simulation, after subtraction of the $^{\cdot}CH_2CONH_2$ features, revealed a major reduction in the yield of T^- with little

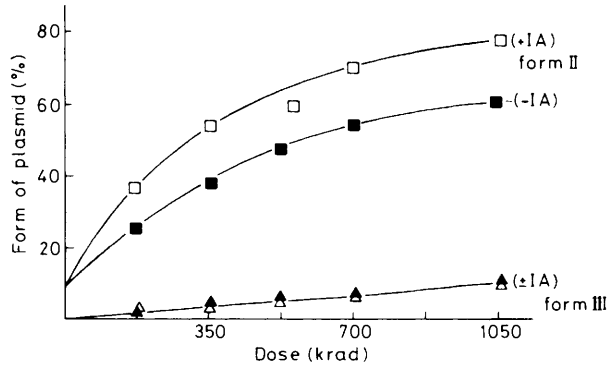


Figure 3. The effect of iodoacetamide on strand-breaks induced by γ-irradiation of plasmid DNA (pBR 322) at 77 K. The percentage of Form II indicates single strand-breaks produced in the presence (10mM) (□) and absence (■) of iodoacetamide. Double strand-breaks formed in the presence (10mM) (△) and absence (▲) of iodoacetamide are indicated by Form III

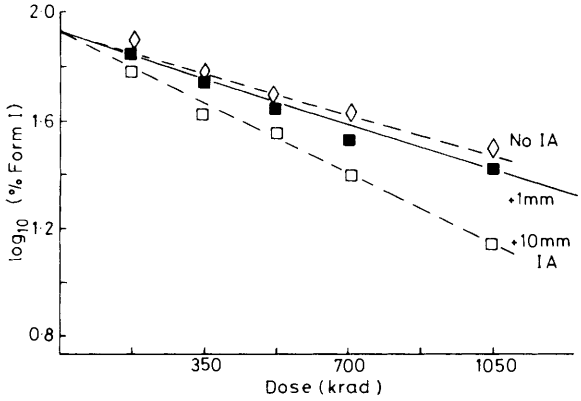


Figure 4. Semi-log plot of single strand-breaks induced in plasmid DNA (pBR 322) following irradiation with ^{60}Co γ-rays at 77 K in the absence (◇) and in the presence of iodoacetamide at 1mM (■) and 10mM (□)

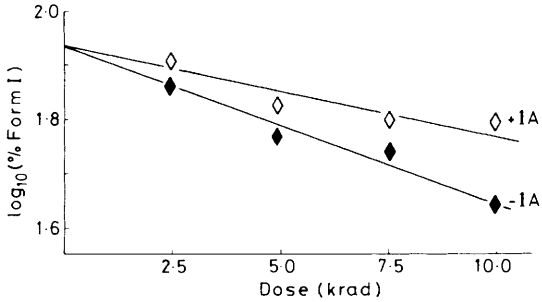


Figure 5. Semi-log plot of single strand-breaks induced in plasmid DNA (pBR 322) following irradiation at room temperature in the presence (10mM) (◇) and absence (◆) of iodoacetamide

change in the yield of G^+. Furthermore, the distinctive octet of $TH^\bullet$ was not detected on annealing to *ca.* 250 K, in marked contrast to the normal reaction.

The e.s.r. spectra of DNA plus iodoacetamide, after irradiation, gave no features assignable to iodine-containing radicals (these would have been easily detected by the large iodine hyperfine coupling). In contrast, when iodoacetamide was irradiated as an aqueous methanol glass, a radical with a large hyperfine coupling from ^{127}I was detected in addition to the $^\bullet CH_2CONH_2$ radicals. This species is also produced on irradiation of solid iodoacetamide[14,15] and is probably the parent radical anion (IA^-). It is curious that the anion is not detected in the DNA system and that dissociation is apparently complete. This may be a general solvation effect, since it is difficult to imagine why DNA should specifically promote dissociation.

On annealing the DNA–iodoacetamide samples to *ca.* 190 K, the asymmetric triplet features disappeared with simultaneous appearance of features assigned to radicals α, β, γ, and δ (Figure 2). The same spectra were observed when deuteriated iodoacetamide was used, indicating that these radicals are not derived from the iodoacetamide and therefore are presumably DNA radicals. In oxygenated DNA samples the e.s.r. spectra were initially unchanged. However, on annealing, the normal growth of signals for the $RO_2^\bullet$ radicals was reduced by the presence of the additive.

Strand-break Studies.—Our assay for strand breaks has been described in detail elsewhere.[9,10] The superhelical Form I of the plasmid pBR 322 can be isolated in good yield and purity from *E. coli.* The introduction of a single strand-break converts this into Form II of the plasmid, the open circular form. A double strand-break gives rise to the linear Form III. The easy separation of these three forms by gel electrophoresis provides a simple and accurate assay for chain scissions introduced by ionising radiation.

The overall results are summarised in Figure 3 and Table 1. The presence of iodoacetamide (10mM) causes an increase in single strand-breaks while the proportion of double strand-breaks remains largely unchanged. The semi-log plot of the disappearance of Form I as a function of the dose is linear and is dependent on the iodoacetamide concentration (Figure 4). For the purpose of comparing the effects of various additives we have used the slopes of these plots to define a parameter which we call the additive effect ratio (a.e.r.). This is defined as the ratio of the slope of the plot for the experiment in the presence of the additive to the slope of the plot for the control experiment where no additive is present. (In these experiments the control experiment consisted of irradiation of DNA samples under ambient atmosphere, in the absence of iodoacetamide.) An a.e.r. of greater than unity corresponds to an *enhancement* of the irradiation dependent damage to DNA due to the additive, *i.e. sensitisation*, while an a.e.r. of less than unity corresponds to a *reduction* in damage, *i.e. protection.* The a.e.r. is clearly dependent on the concentration of the additive. Irradiation of plasmid DNA at 77 K under ambient conditions with an iodoacetamide concentration of 1mM revealed an a.e.r. of 1.22 and at 10mM-iodoacetamide this rose to 1.67. With 10mM-iodoacetamide in deoxygenated samples the a.e.r. decreased to 1.17 while in fully oxygenated samples an increase to 1.97 was detected (Table 2).

For comparison, we have irradiated aqueous solutions of the plasmid in the presence and absence of 10mM-iodoacetamide at room temperature. In contrast with our studies at 77 K, the principal mode of damage is indirect, mediated through $OH^\bullet$ radicals and solvated electrons. Under these conditions, 10mM-iodoacetamide protected the DNA against overall strand-breaks, as can be judged from the a.e.r. of 0.46 (Figure 5, Table 3).

Discussion

Radical Yields.—The primary effect of this additive is to capture electrons, in competition with electron capture by thymine, thereby reducing the yield of T^-. We have observed a similar effect in studies on frozen aqueous solutions of DNA

Table 1. Percentage form of the plasmid (pBR 322) following irradiation at 77 K under the conditions shown

Dose* (krad)	Under ambient conditions		Plus 10mM-IA	
	% Form II	% Form III	% Form II	% Form III
0	10.1	0	10.1	0
175	26.1	2.5	36.8	2.4
350	37.1	3.9	53.0	3.6
525	47.7	5.3	59.5	5.7
700	54.2	6.6	68.9	6.4
1 050	60.1	10.6	76.7	9.5

* Non-S.I. units employed: 1 rad = 10^{-2} J kg^{-1}; 1 G = 10^{-4} T; 1 eV ≈ 1.60 × 10^{-19} J.

Table 2 $\log_{10}$ (% Form I) following irradiation of plasmid DNA (pBR 322) at 77 K in the presence of iodoacetamide under ambient conditions

Dose* (krad)	$\log_{10}$ (% Form I)				
	DNA Control	+1mM-IA	+10mM-IA ambient	+10mM-IA oxygen	+10mM-IA nitrogen
0	1.953	1.953	1.953	1.953	1.953
175	1.853	1.802	1.784		
350	1.771	1.730	1.637		
525	1.672	1.609	1.542		
700	1.593	1.489	1.393	1.283	1.533
1 050	1.467	1.360	1.140	1.017	1.393
a.e.r.		*1.21*	*1.62*	*1.97*	*1.16*

* As for Table 1 above.

Table 3. $\log_{10}$ (% Form I) following irradiation of plasmid DNA (pBR 322) at room temperature in the presence of iodoacetamide

Dose* (krad)	$\log_{10}$ (% Form I)	
	Control DNA	+10mM-IA
0	1.953	1.953
2.5	1.858	1.895
5.0	1.774	1.839
7.5	1.728	1.803
10.0	1.635	1.789
a.e.r.		*0.54*

* As per Table 1.

doped with metronidazole (MET).[10] Iodoacetamide had little effect on the yield of the other base radical G^+. The observation that electrons can be trapped by the additive without leading to a significant increase in total radical yield implies that electron return is not important in our system, in keeping with our earlier studies.

We have previously shown that under conditions where only G^+ and T^- are initially detected by e.s.r., strand breaks persist at a significant level.[9,10] This suggests that these radicals are capable of initiating a reaction sequence that ultimately leads to strand breaks. This pathway is yet to be fully defined. However, it is reasonable to assume that at some stage the radical centre must be transferred to the sugar moiety. Solution studies have established the β-elimination mechanism[16] (Scheme). Furthermore, Schulte-Fröhlinde and his co-workers[17] have recently reported that base–peroxyl radicals can give rise to strand breaks *via* hydrogen-atom abstraction from the 4′-position of the sugar residue of the neighbouring nucleoside (Scheme). Although the indirect damage mechanism, as studied by these workers, and our own studies on the direct damage mechanism are entirely different, we can detect the formation of base–peroxyl radicals by e.s.r.[9] and these can presumably also react as shown in the Scheme. With the exception of radicals α, β, γ, and δ, we have not thus far been able to detect any other possible intermediates along this pathway, presumably because they react further at the temperatures at which they are formed, thus only attaining low stationary concentrations.

Iodoacetamide appears to compete for electron capture, giving rise to reduced yields of DNA radicals initially, as has been observed previously for metronidazole.[10] Iodoacetamide ultimately gives rise to *increased* damage to DNA when irradiated at 77 K, in marked contrast to our previous observations with MET where a reduction in yield of DNA radicals also gave rise to a decrease in yield of strand breaks. We suggest that this difference is associated with the difference in reactivities of the radials derived from the additives themselves. The $^{\bullet}CH_2CONH_2$ radicals that we detect by e.s.r. would be expected to be fairly reactive. We suggest that a significant reaction is hydrogen-atom abstraction from the sugar moiety [reaction (2)]. At least some of these sugar radicals proceed on to strand breaks and if this process is relatively efficient then this would explain the enhanced damage to DNA in the presence of iodoacetamide.

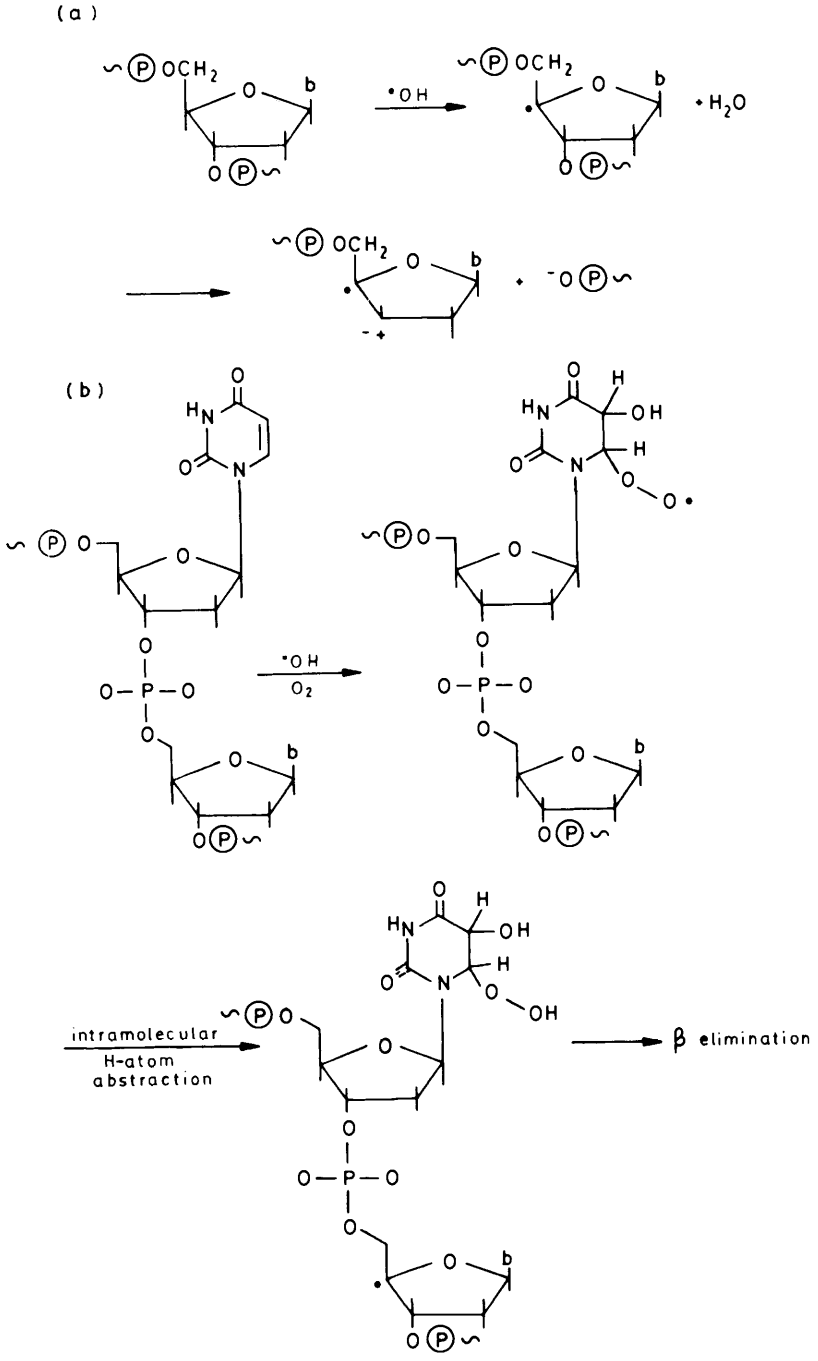
$$^{\bullet}CH_2CONH_2 + \text{Sugar-H} \longrightarrow CH_3CONH_2 + \text{Sugar}^{\bullet} \longrightarrow \text{Strand Breaks} \quad (2)$$

Identification of Secondary DNA Radicals.—Directly after loss of the $^{\bullet}CH_2CONH_2$ radicals, spectra of the type shown in Figure 2a were obtained. We suggest this is due to at least two species, one with a total splitting of *ca.* 90 G (outer features α) and another species β giving a quartet, for which the outer lines display marked asymmetry. The initial decay of these features is uniform, as indicated, and therefore we consider that a single species dominates initially. On further annealing (Figure 2b) central features appear, which may be part of a set of four lines (γ) and there is yet another species characterised by a doublet of *ca.* 30 G splitting (δ). The feature for γ were lost prior to this doublet (δ) which proved to be the most persistent of all the secondary radicals.

These results are reminiscent of those obtained for radicals formed from deoxyribose by hydrogen-atom abstraction by free hydrogen atoms.[18] In particular, the quartet β closely resembles a species labelled Q, the outer lines of α correspond to a species labelled Q′, and the 30 G doublet is identical to a 30 G doublet which was the last species to be lost on annealing in the work on deoxyribose. This comparison strongly supports our suggestion that the present species are sugar radicals. There are five reasonable sugar radicals that might be formed by attack of $^{\bullet}CH_2CONH_2$, *viz.* (**I**)—(**V**).

Unfortunately, it is difficult to predict the precise form of the e.s.r. spectra for any of these radicals, since in all cases there are β-protons, whose hyperfine coupling is a function of the twist angle, θ. This depends closely on the initial conformation of the ribose entity and on changes that occur on relaxation following hydrogen-atom abstraction. However, general statements can be made.

Species (α) must have coupling to at least three protons, so radicals (**II**)—(**IV**) are possible candidates. In our previous study, radical (**II**) was favoured, but this is not compelling, and in fact radical (**II**) is chemically the least reasonable. The dominant species (β) appears to require coupling to three nearly equivalent protons, the asymmetry of the outermost lines suggesting the presence of a $^{\bullet}CH_2$–R unit. Unfortunately, no

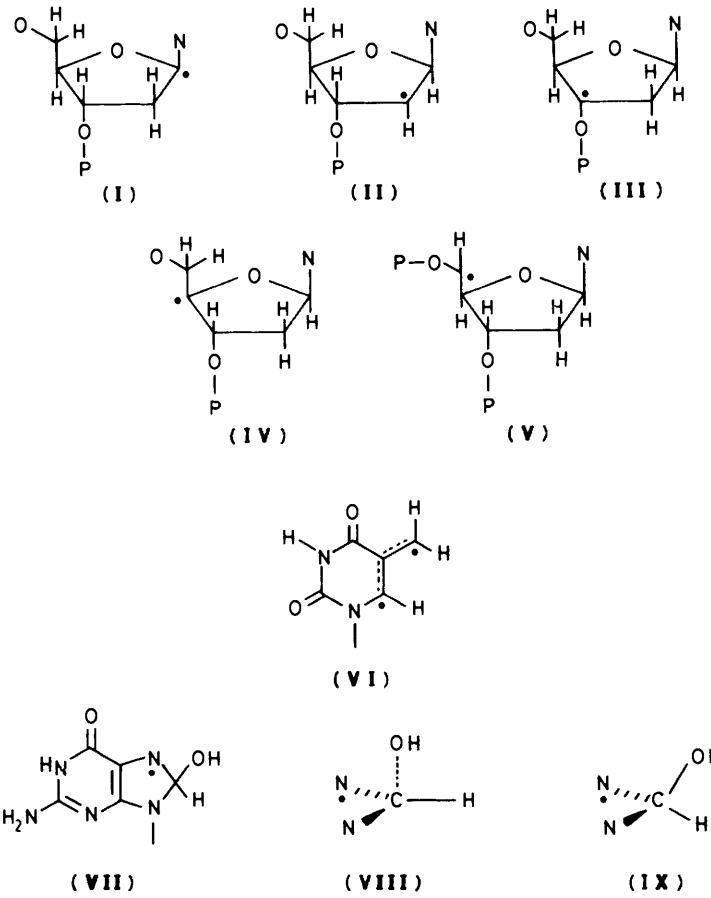


Scheme.

such species can be formed by hydrogen-atom abstraction from the sugar moiety. Also, the average coupling of *ca.* 15 G is too small for such a unit. If this asymmetry is ignored, or assigned to the presence of overlapping features from other radicals, then radical (**IV**) is possible provided θ *ca.* 60° for all three β protons, which is impossible for the other radicals (**I**)—(**III**) and (**V**). This is the same as the previous assignment. Finally, the 30 G doublet could be from radical (**V**), provided θ *ca.* 90° so that the β proton coupling is small. This also requires that $A(^{31}P)$ be small, which is usually the case for such radicals. (This coupling is also strongly θ dependent.)

In view of the results for deoxyribose,[18] we think that our assignments to sugar radicals are most reasonable. However, two other radicals should be considered. One is radical (**VI**), formed by hydrogen-atom abstraction from the methyl group of thymine. This is an allylic-type radical, which, provided the SOMO resembles that indicated in (**VI**), should exhibit coupling of *ca.* 14 G to three nearly equivalent protons. This assignment nicely accommodates the anisotropy observed in the features of species β and seems to us to be quite reasonable. At present we have no way of distinguishing between this and a sugar-centred species. However, it is unlikely that radical (**VI**) would lead efficiently to strand breaks and therefore would not account for our observed sensitisation. We therefore favour the assignment to sugar radicals.

Finally we should consider the possibility that one of these



radicals could be 'G–OH (VII). As discussed previously 'G–OH is a probable product from G^+ on annealing, although it is not usually detected. However, although the outer features for β are in accord with expectation for an anisotopic coupling to ^{14}N, this radical normally exhibits a coupling of 33 G to a single proton, which is far too large for species β. However, if the initial adduct was prevented from adopting tetrahedral geometry, perhaps by solvation, then the distorted structure would exhibit a smaller proton coupling. In structure (VIII) the coupling between the radical and the proton would be small because the hydrogen is close to the nodal plane of the electron. However, as the OH group and the proton move towards structure (IX) the coupling should increase to 33 G. If this hypothesis were correct then an increase in temperature would be expected to alter the H hyperfine coupling as the system becomes mobile and the structure becomes more tetrahedral. This was not observed. Furthermore, it is not clear why GOH' formation should be detectable only in the presence of iodoacetamide, since its formation from G^+ and water should be independent of the additive.

Temperature Profile.—We have also monitored the temperatures at which various radicals decay. It is only possible to measure a temperature range (rather than a precise temperature) over which radicals signals are lost, since the system is relatively heterogeneous. The results are compared with those obtained for the same DNA systems in the absence of iodoacetamide. The most important aspect of these results is that radicals α, β, γ, and δ are all lost at 249 ± 5 K which is significantly below those at which the DNA radicals normally decay (267 ± 5 K). This helps to justify our postulate that DNA radicals detectable by e.s.r. spectroscopy decay *via* the formation of sugar radicals even though they are not detected. The above result suggests that at the temperatures at which the primary radicals normally decay, the intermediate sugar radicals react rapidly and thus these secondary radicals do not accumulate to concentrations sufficient for detection. It should be noted that all the detectable radicals have reacted *before* the systems becomes fluid.

Room-temperature Studies.—In previous room-temperature radiation studies in which iodoacetamide was added to *in vivo* systems, iodine atoms were postulated as a short-lived transient responsible for the observed radiosensitisation.[19] Formation of I' from I^- in the presence of OH' has been shown to occur during the irradiation of *in vivo* systems[20,21] [reaction (3)]

$$I^- + OH' \rightleftharpoons IOH^- \rightleftharpoons I' + OH^- \qquad (3)$$

together with further iodine transients detected by pulse radiolysis.[22] In our frozen systems, since I^- is formed in the primary radiation-dependent step [equation (1)], secondary processes that might convert I^- into I' seem to us to be improbable. Furthermore, no iodine radicals were detected by e.s.r. spectroscopy in our DNA–iodoacetamide systems. Other *in vitro* studies have shown a reduction in the number of strand breaks (*e.g.* in lysed phage cells[7]) in agreement with our room-temperature radiation studies. This protection effect under conditions of indirect radiation damage is easily understood in terms of the known ability of iodoacetamide to scavenge OH' radicals[21] and solvated electrons.[23] Since the major contribution to DNA damage in dilute solution is *via* hydroxyl radicals, the protection effect of iodoacetamide that we observe when irradiating at room temperature is easily understood in terms of

replacing these highly reactive radicals by iodine atoms as in equation (4). The latter radicals we would expect to be considerably less reactive.

$$^{\bullet}OH + ICH_2CONH_2 \longrightarrow {}^{\bullet}I + CH_2(OH)CONH_2 \quad (4)$$

It is important to note that the apparently large doses required in the frozen aqueous studies as compared with the room-temperature studies merely reflects the different effective sample sizes. In the dilute aqueous system a considerable amount of the initial damage to water (which represents the vast majority of the sample) ends up as DNA damage. In the direct damage mechanism, only the ionisations in DNA itself contribute to the chemical damage, giving a considerably smaller effective target.

Double Strand-breaks.—In Part 1 we suggested that the remarkably high relative yield of double strand-breaks in our studies arises because electron-loss and electron-gain centres are formed initially close together.[9] We suggested that if a significant proportion of G^+–T^- pairs are trapped in opposite strands between *ca.* 15 and 30 Å and providing both primary radicals can give rise to strand breaks, this would provide a good explanation of the high numbers of double strand-breaks in our system. In the presence of iodoacetamide, T^- centres are replaced by $^{\bullet}CH_2CONH_2$ radicals. These can diffuse on annealing and we suggest that they react by abstracting hydrogen atoms from DNA. The enhanced damage in terms of single strand-breaks must be due to the efficiency of this hydrogen-atom abstraction. The fact that iodoacetamide does not cause a similar increase in double strand-breaks can be explained in terms of our original proposal for how they arise if one assumes that there is a reasonable chance that the $^{\bullet}CH_2CONH_2$ radicals, being mobile, can diffuse away from the original site of electron loss (G^+) before reacting. This will result in more single strand-breaks and fewer double strand-breaks (Figure 3).

Conclusions.—We conclude that, under our conditions of phase separation, iodoacetamide molecules are trapped in the frozen matrix close to DNA molecules. On irradiation, ejected electrons are captured by iodoacetamide preferentially either directly or by electron transfer from initially formed T^- radicals. This leads to a major loss of T^-, and an almost complete suppression of TH$^{\bullet}$ formation. We explain our observation of increased damage to DNA on irradiation at 77 K in the presence of iodoacetamide in terms of the reactivity of the $^{\bullet}CH_2CONH_2$ radicals. On annealing, we detect a conversion of these radicals into other secondary radicals which we suggest are primarily sugar radicals. In contrast to this we have demonstrated that when DNA is irradiated at room temperature, damage is reduced by the presence of iodoacetamide, presumably because iodoacetamide is an efficient scavenger of both $^{\bullet}OH$ radicals and electrons.

Acknowledgements

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