Free-radical-induced formation of an 8,5'-cyclo-2'-deoxyguanosine moiety in deoxyribonucleic acid

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Isolation and identification of a novel 'OH-induced product, namely an 8,5'-cyclo-2'-deoxyguanosine moiety, in DNA and 2'-deoxyguanosine are described. 'OH radicals were generated in dilute aqueous solutions by γ -irradiation. Analyses of 2'-deoxyguanosine and enzymic hydrolysates of DNA by gas chromatography–mass spectrometry (g.c.–m.s.) after trimethylsilylation showed the presence of 8,5'-cyclo-2'-deoxyguanosine on the basis of its fragment ions. This product was isolated by h.p.l.c. Its u.v. and n.m.r. spectra taken were in agreement with the structure suggested by its mass spectrum. Exact masses of the typical ions from the mass spectrum of the trimethylsilyl derivative of this product were measured by high-resolution m.s. The values found were in excellent agreement with the theoretical mass derived from the suggested fragmentation patterns. Both (5'R)- and (5'S)-epimers of 8,5'-cyclo-2'-deoxyguanosine were observed. These two diastereomers were separated from each other by g.c. as well as by h.p.l.c. The assignment of the epimers was accomplished on the basis of the n.m.r. data. The formation of 8,5'-cyclo-2'-deoxyguanosine was suppressed by the presence of O₂ in the solutions. The use of g.c.–m.s. with the selected-ion monitoring technique facilitated the detection of 8,5'-cyclo-2'-deoxyguanosine in DNA at radiation doses as low as 1 Gy. Its mechanism of formation probably involves hydrogen atom abstraction by 'OH radicals from the C-5' of the 2'-deoxyguanosine moiety followed by intramolecular cyclization with the formation of a covalent bond between the C-5' and C-8 and subsequent oxidation of the resulting N-7-centred radical.

INTRODUCTION

Damage to DNA induced by free radicals, e.g. 'OH, appears to play an important role in mutagenesis, carcinogenesis and aging (Cerutti, 1985). In living cells, free radicals are generated by cellular metabolism or external agents such as ionizing radiations (Halliwell & Gutteridge, 1985). Free radicals create a number of lesions in DNA involving single-strand and double-strand breaks, altered bases, base-free sites and cross-linking between DNA and proteins (Teoule & Cadet, 1978; Mee & Adelstein, 1981; Scholes, 1983).

Lesions produced in DNA, however, are subject to repair processes and, unless repaired, may have detrimental biological consequences (Friedberg, 1984). Elucidation of the chemical nature of free-radical-induced DNA lesions is therefore necessary for the assessment of their biological consequences and repair.

Reactions of ionizing-radiation-generated free radicals with DNA and its constituents have been investigated quite extensively. A large number of products of both pyrimidine and purine bases have been isolated and identified (for reviews see Teoule & Cadet, 1978; Cadet & Berger, 1985). Free-radical-induced products of the 2'-deoxyribose moiety in DNA have also been described (Dizdaroğlu *et al.*, 1975; Beesk *et al.*, 1979). In the case of purine nucleosides and nucleotides, one unique reaction induced by 'OH radicals is the intramolecular cyclization between the C-5' of the sugar moiety and the C-8 of the purine ring. This reaction and the resulting product, i.e. 8,5'-cyclo-AMP, was first described by Keck (1968) in AMP irradiated in the absence of O₂. Subsequent reports dealt with the quantification and the

pH-dependence of the 8,5'-cyclo-AMP production (Raleigh *et al.*, 1976; Raleigh & Fuciarelli, 1985) and the formation of the analogous product from dAdo, i.e. 8,5'-cyclo-dAdo (Mariaggi *et al.*, 1976). This reaction has been reported as highly stereoselective as a result of the identification of one of the two possible 5'-epimers of 8,5'-cyclo-dAdo (for a review see also Cadet & Berger, 1985). Recently, immunochemical detection of 8,5'-cyclo-Ado in poly(A) and 8,5'-cyclo-dAdo in DNA γ -irradiated in the absence of O₂ was described (Fuciarelli *et al.*, 1986).

Berger & Cadet (1983) reported the 'OH-induced formation of 8,5'-cyclo-2',5'-dideoxyguanosine as a minor product in aqueous solutions of dGuo and dGMP. On the other hand, the same paper and a later review article by the same authors (Cadet & Berger, 1985) reported the lack of the detectable formation of 8,5'-cyclo-dGuo from dGuo, which is the analogous product to 8,5'-cyclo-dAdo from dAdo or 8,5'-cyclo-AMP from AMP.

The present paper describes the isolation and characterization of an 8,5'-cyclo-dGuo moiety from both dGuo and DNA exposed to radiation-generated 'OH radicals in aqueous solution. Capillary g.c.–m.s., h.p.l.c., u.v. spectroscopy, n.m.r. spectroscopy, s.i.m. and exact mass measurement by m.s. were used for this purpose.

MATERIALS AND METHODS

Materials

Calf thymus DNA, dGuo and bis(trimethylsilyl)tri-fluoroacetamide were purchased from Sigma Chemical

Abbreviations used: Ado, adenosine; dGuo, 2'-deoxyguanosine; dAdo, 2'-deoxyadenosine; g.c.–m.s., gas chromatography–mass spectrometry; s.i.m., selected-ion monitoring; Gy, gray (= 1 J/kg = 100 rad); a.m.u., atomic mass unit.

Co. Acetonitrile was obtained from Pierce Chemical Co. Water purified through a Millipore system was used for all purposes. All enzymes used were purchased from Boehringer Mannheim. Thymidine glycol was isolated by h.p.l.c. from OsO_4 -treated thymidine. Samples of (*R*)- and (*S*)-8,5'-cyclo-Ado were kindly provided by Dr. J. A. Raleigh of the Cross Cancer Institute, Edmonton, Alberta, Canada.

Irradiations

Aqueous solutions of DNA (0.25 mg/ml) and dGuo (1 mM) were saturated with N_2O , $\text{N}_2\text{O}/\text{O}_2$ (1:1), N_2 or air for 30 min and irradiated in a ^{60}Co γ -radiation source (dose range 80–400 Gy; dose rate 80 Gy/min). For experiments at low doses below 10 Gy, a dose rate of 1 Gy/min was used. After irradiation, the samples were freeze-dried.

Enzymic hydrolysis

A 1 mg portion of DNA samples was dissolved in 0.5 ml of 10 mM-Tris/HCl buffer, pH 8.5, containing 10 mM- MgCl_2 . This solution was incubated with deoxyribonuclease I (20 units), spleen exonuclease (0.004 unit), snake-venom exonuclease (0.002 unit) and alkaline phosphatase (2.5 units) for 24 h at 37 °C. Then the sample was freeze-dried and used for g.c.-m.s. analysis.

Trimethylsilylation

Samples were trimethylsilylated in polytetrafluoroethylene-capped hypovials (Pierce Chemical Co.) with 0.2 ml of a bis(trimethylsilyl)trifluoroacetamide/acetonitrile (1:1, v/v) mixture by heating for 15 min at 130 °C.

G.c.-m.s.

A Hewlett-Packard model 5880A microprocessor-controlled gas chromatograph interfaced to a Hewlett-Packard model 5970A mass selective detector was used. The injection port and g.c.-m.s. interface were both maintained at 250 °C and the ion source at approx. 200 °C. Separations were carried out in a fused silica capillary column (12 m $\times$ 0.2 mm internal diam.) coated with cross-linked SE-54 (5% phenylmethyl silicone; film thickness, 0.11 μm) (Hewlett-Packard). He was used as the carrier gas at an inlet pressure of 100 kPa. The split ratio was 20:1. Mass spectra were obtained at 70 eV.

H.p.l.c.

Separations were carried out on a Hewlett-Packard model 1090 microprocessor-controlled liquid chromatograph equipped with a model HP 1040A high-speed spectrophotometric detector and a Supelcosil LC-8-DB column (25 cm $\times$ 1 cm; particle size 5 μm ; Supelco).

Exact mass measurement

A VG analytical model 70E-HF mass spectrometer was used at a resolution of 5000. The scans were taken in a linear up mode with the field control at 10 s/scan. The data were acquired in continuum mode as single scans, with perfluorokerosene being let in as the reference. The ion-source temperature was 210 °C.

N.m.r. spectroscopy

^1H -n.m.r. spectra were recorded on a Varian XL-200 n.m.r. spectrometer at 200 MHz at 21 °C, with tetramethylsilane as a reference compound. Approx. 1 mg of the sample was dissolved in 0.5 ml of $[\text{D}_6]\text{H}_2\text{O}$ /acetone-

Fig. 1. Total-ion chromatogram obtained from a trimethylsilylated sample of dGuo γ -irradiated in N_2O -saturated solution (dose 400 Gy)

The column was a fused silica capillary coated with SE-54 (12 m $\times$ 0.2 mm internal diam.), programmed from 190 to 250 °C at 7 °C/min after 3 min at 190 °C. For other details see the Materials and methods section. Peak assignments were as follows: peaks 1 and 2, two diastereomers of 8,5'-cyclo-dGuo-(Me_3Si)₄; peak 3, dGuo-(Me_3Si)₄; peak 4, 8-hydroxy-dGuo-(Me_3Si)₅; peak 5, dGuo-(Me_3Si)₆.

$[\text{D}_6]\text{H}_2\text{O}$ /acetone/ $[\text{D}_6]\text{H}_2\text{O}$ (5:3:1, by vol.) for this purpose.

RESULTS

Samples of dGuo and enzymic hydrolysates of DNA were trimethylsilylated and analysed by g.c.-m.s. in order to find out whether the product analogous to those found in irradiated AMP and dAdo (see the Introduction) was present in γ -irradiated dGuo and DNA. The g.c.-m.s. technique has been shown previously to be an excellent analytical tool for characterization of free-radical-induced base damage in DNA (Dizdaroglu, 1984, 1985a,b; Dizdaroglu & Bergtold, 1986). Figs. 1 and 2 show total-ion chromatograms obtained from a trimethylsilylated sample of γ -irradiated dGuo and a trimethylsilylated enzymic hydrolysate of γ -irradiated DNA respectively. The expected molecular ion (m/z 553) of the trimethylsilyl derivative of 8,5'-cyclo-dGuo [8,5'-cyclo-dGuo-(Me_3Si)₄] was monitored simultaneously by the use of single-ion monitoring (not shown here) and found to be present in the mass spectra of the compounds represented by peaks 1 and 2 in Fig. 1 and by peaks 7 and 8 in Fig. 2. The mass spectra taken from these peaks were all identical with one another. One of them is illustrated in Fig. 3. This spectrum could be interpreted in part on the basis of the well-known fragmentation patterns of the trimethylsilyl derivatives of nucleosides (McCloskey,

Fig. 2. Total-ion chromatogram obtained from a trimethylsilylated enzymic hydrolysate of DNA γ -irradiated in N_2O -saturated solution (dose 400 Gy)

Column details were as indicated in Fig. 1 legend. Peak assignments were as follows: peaks 1 and 2, two diastereomers of thymidine glycol- $(Me_3Si)_5$; peak 3, 2'-deoxyinosine- $(Me_3Si)_3$ (this compound probably was formed by deamination of dAdo under the hydrolysis conditions used here); peak 4, dAdo- $(Me_3Si)_3$; peak 5, 8-hydroxy-2'-deoxyinosine- $(Me_3Si)_4$ (this compound probably was formed by deamination of 8-hydroxy-dAdo, which is a known product in γ -irradiated DNA); peak 6, 4-amino-5-formylamino-6-(2'-deoxyribose)aminopyrimidine- $(Me_3Si)_5$; peaks 7 and 8, two diastereomers of 8,5'-cyclo-dGuo- $(Me_3Si)_4$; peak 9, dGuo- $(Me_3Si)_4$; peak 10, 8-hydroxy-dGuo- $(Me_3Si)_5$; peak 11, 2-amino-4-hydroxy-5-formylamino-6-(2'-deoxyribose)aminopyrimidine- $(Me_3Si)_5$.

Fig. 3. One of the identical mass spectra taken from peaks 1 and 2 in Fig. 1 and peaks 7 and 8 in Fig. 2

1974; Pang *et al.*, 1982). The ion of highest mass at m/z 553 in Fig. 3 was attributed to the molecular ion (M^+) and the ion at m/z 538 to a characteristic ($M-Me$)⁺ ion. The ion at m/z 448 corresponds to the loss of trimethylsilanol (Me_3SiOH ; 90 a.m.u.) from m/z 538. The most intense ion at m/z 397 probably contains the base plus portions of the sugar moiety as shown below:

The transfer of two hydrogen atoms are necessary for the formation of the m/z -397 ion. Ions with base plus portions of the sugar moiety and hydrogen transfers are common to trimethylsilyl derivatives of nucleosides (McCloskey, 1974; Pang *et al.*, 1982). The ion containing the base and the same sugar portion but formed without hydrogen transfer was also observed at m/z 395. The intense ion at m/z 380 corresponds to elimination of a methyl group from m/z 395. Another abundant ion containing base plus portions of the sugar moiety was observed at m/z 422 [base-C(1')H-C(2')H₂-C(5')-HOSiMe₃; see also above]. The ion at m/z 366 appears to be formed by transfer of a trimethylsilyl group to the base (base + Me_3Si ion), which is a common process for trimethylsilyl-nucleosides (Pang *et al.*, 1982). The ion of low abundance at m/z 524 can be attributed to elimination of HC(4') O from M^+ . The absence of the m/z -103 ion in Fig. 3 is noteworthy. This ion ($Me_3Si\dot{O}=CH_2$) occurs extensively in the mass spectra of trimethylsilyl derivatives of nucleosides and was assigned mostly to the C(5')-silyloxy group (McCloskey, 1974; Pang *et al.*, 1982). Its absence in Fig. 3 is in excellent agreement with the cyclic structure involving the C-5' of the sugar moiety as shown above. Ions at m/z 73 and 147 are commonly observed with trimethylsilyl derivatives and serve no diagnostic purpose.

Support for the structure and fragmentation patterns discussed above was obtained from the mass spectrum of the trimethylsilyl derivative of (5'*S*)-8,5'-cyclo-Ado, which was available for analysis. Fig. 4 shows the mass spectrum of 8,5'-cyclo-Ado-(Me_3Si)₄, which has the same M_r as 8,5'-cyclo-dGuo-(Me_3Si)₄. M^+ and ($M-Me$)⁺ ions were observed at m/z 553 and 538 respectively. The intense ion at m/z 422 was attributed to the analogous fragmentation leading to the m/z -422 ion in the mass spectrum of 8,5'-cyclo-dGuo-(Me_3Si)₄. The main difference between these two spectra is the absence of the m/z -397 ion in Fig. 4. Instead, an intense ion at m/z 309 was observed, which represents a mass difference of 88 a.m.u. from m/z 397. This is expected because of the absence of a trimethylsilyloxy group in the purine ring of 8,5'-cyclo-Ado-(Me_3Si)₄. The ions at m/z 292 and 278

in Fig. 4 are analogous to the ions at m/z 380 and 366 in Fig. 3 respectively. Another important difference between the two spectra is the presence of an abundant ion at m/z 217 in Fig. 4, which is typical of trimethylsilyl derivatives of ribonucleosides (McCloskey, 1974). Again, the absence of the m/z -103 ion in Fig. 4 clearly indicates the involvement of the C-5' of the sugar moiety in the cyclic structure:

The presence of two compounds with identical mass spectra (peaks 1 and 2 in Fig. 1, and peaks 7 and 8 in Fig. 2) can be attributed to the two possible diastereomers [(5'*R*) and (5'*S*)] of 8,5'-cyclo-dGuo. These isomers could not be distinguished from each other by m.s. However, the isomer eluted first can be assigned to the (*R*)-epimer and the isomer eluted later to the (*S*)-epimer. This assignment is based on the g.c. elution order of the two available diastereomers of 8,5'-cyclo-Ado, where the (*R*)-epimer is eluted before the (*S*)-epimer under the same g.c. conditions. The origins of other numbered peaks in Figs. 1 and 2 are given in the Figure captions.

In order to obtain further structural information on the product discussed above, an attempt was made to isolate it by h.p.l.c. Fig. 5 illustrates a chromatogram obtained from a sample of γ -irradiated dGuo. Peaks 4 and 5 correspond to dGuo and 8-hydroxy-dGuo respectively. The product represented by peak 1 exhibits an absorption spectrum (insert A) quite similar to that of dGuo (insert B), with the only difference being the shift of the absorption maximum by about 5 nm into a longer wavelength (the h.p.l.c. instrument used is capable of taking absorption spectra during an analysis). The same spectral behaviour has been observed previously for 8,5'-cyclo-AMP when compared with AMP (Keck, 1968). The product represented by peak 1 in Fig. 5 was collected and analysed by g.c.-m.s. after removal of the eluent and subsequent trimethylsilylation. A single g.c. peak was obtained with the same retention time as peaks 1 and 7 in Figs. 1 and 2 respectively, and a mass spectrum identical with that illustrated in Fig. 3. Consequently, peak 1 in Fig. 5 was assigned to (*R*)-8,5'-cyclo-dGuo. The (*S*)-epimer was found to be co-eluted with another unidentified product as peak 3 in Fig. 5. The elution order of the two diastereomers of 8,5'-cyclo-dGuo was in accord with that of the (*R*)- and (*S*)-epimers of 8,5'-cyclo-Ado under the same h.p.l.c. conditions.

The trimethylsilyl derivative of the product collected by h.p.l.c. (peak 1 in Fig. 5) was analysed by high-resolution m.s. with exact mass measurements in order to ascertain the fragmentation pathways suggested

Fig. 4. Mass spectrum of (S) -8,5'-cyclo-Ado-(Me₃Si)₄

Fig. 5. Liquid chromatogram obtained from a sample of dGuo γ -irradiated in N₂O-saturated solution (dose 400 Gy)

The column was Supelcosil LC-8-DB (25 cm $\times$ 1 cm; particle size 5 μ m), at ambient temperature. Gradient elution was performed at a rate of 0.4% (v/v) acetonitrile in water/min. The flow rate was 4 ml/min. Inserts A and B show the u.v. spectra taken from peaks 1 and 4 respectively. Peak assignments were as follows: peak 1, (R) -8,5'-cyclo-dGuo; peak 2, guanine; peak 3, (S) -8,5'-cyclo-dGuo and an unidentified product; peak 4, dGuo; peak 5, 8-hydroxy-dGuo.

above. Exact masses of characteristic ions of high abundance at m/z 553, 538, 422, 397 and 380 were measured. Table 1 clearly demonstrates the excellent agreement of the obtained values with the theoretical masses calculated on the basis of the compositions of the fragments suggested above.

In agreement with the structural evidence from g.c.-m.s., u.v. spectroscopy and exact mass measurements, the ^1H -n.m.r. spectrum of the product isolated by h.p.l.c. (peak 1 in Fig. 5) showed no signal from the proton at

C-8 of the purine moiety, which is lost in the cyclization process. The n.m.r. characteristics of this product are given in Table 2. These data are quite similar to those published for 8,5'-cyclo-dAdo (Mariaggi *et al.*, 1976). The small coupling observed between H-4' and H-5' (Table 2) is indicative of the (R) -epimer, where the dihedral angle between these protons is close to 90° (Mariaggi *et al.*, 1976; Raleigh & Blackburn, 1978; Haromy *et al.*, 1980; Raleigh & Fuciarelli, 1985). This assignment by n.m.r. spectroscopy of the product

Table 1. Exact masses of some ions from the mass spectrum in Fig. 3

Ion	Composition	Exact mass	
		Calculated	Measured
M^{+}	$C_{22}H_{43}N_5O_4Si_4$	553.2392	553.2375
$(M-Me)^{+}$	$C_{21}H_{40}N_5O_4Si_5$	538.2157	538.2148
422	$C_{17}H_{32}N_5O_2Si_3$	422.1864	422.1902
397	$C_{16}H_{31}N_5O_2Si_3$	397.1786	397.1764
380	$C_{14}H_{26}N_5O_2Si_3$	380.1394	380.1367

Table 2. N.m.r. characteristics of the product isolated by h.p.l.c. (peak 1 in Fig. 5)

	Proton chemical shifts					
	H-1'	H-2'	H-2''	H-3'	H-4'	H-5'
δ (p.p.m.)	6.30	2.44	2.11*	4.35	4.61	4.69
Multiplicity	d	dd	dt	dd	dd†	d
	Proton coupling constants					
	$J_{1',2'}$	$J_{1',2''}$	$J_{2',2''}$	$J_{2',3'}$	$J_{2'',3'}$	$J_{3',4'}$
J (Hz)	0	4.7	14	7.4	4.2	< 0.6

* Determined in ^2H_2O .
† Partially resolved dd (broad singlet).

represented by peak 1 in Fig. 5 as the (*R*)-epimer of 8,5'-cyclo-dGuo is in accord with that made above on the basis of the g.c. and h.p.l.c. elution orders.

Attempts were also made to identify 8,5'-cyclo-dGuo in DNA at low radiation doses. This was done by the use of g.c.-m.s./s.i.m. This technique has been previously shown to be an excellent tool for characterization of free-radical-induced base damage in DNA at very low concentrations and radiation doses (below 10 Gy) (Dizdaroglu & Bergtold, 1986). In the present work some ten characteristic ions from the mass spectrum of 8,5'-cyclo-dGuo-(Me₃Si)₄ (Fig. 3) were monitored. Figs. 6(a) and 6(b) show s.i.m. plots obtained from trimethylsilylated enzymic hydrolysates of γ -irradiated (5 Gy) and non-irradiated DNA samples respectively. The presence of the recorded ions is clearly seen in Fig. 6(a) at the g.c. retention times characteristic of the diastereomers of 8,5'-cyclo-dGuo-(Me₃Si)₄ (indicated with the arrows in Fig. 6a). As Fig. 6(b) clearly shows, these ions were not present in non-irradiated DNA samples. The area count of each recorded ion was then calculated by the integrator of the instrument by using the tangent-slope method. The acquired data were used subsequently to generate a mass spectrum by setting the area count of the most abundant ion equal to 100% of relative intensity. The partial mass spectrum obtained in this way (Fig. 7) was identical with that of authentic 8,5'-cyclo-dGuo-(Me₃Si)₄ shown in Fig. 3. The presence of 8,5'-cyclo-dGuo in γ -irradiated DNA could be detected in this work at radiation doses as low as 1 Gy by the use of the g.c.-m.s./s.i.m. technique.

The yield of 8,5'-cyclo-dGuo was determined by g.c.

Fig. 6. S.i.m. plot of some characteristic ions of 8,5'-cyclo-dGuo-(Me₃Si)₄ in a trimethylsilylated enzymic hydrolysate of DNA

(a) γ -irradiated in N_2O -saturated solution with a radiation dose of 5 Gy; (b) non-irradiated. Column details were as indicated in Fig. 1 legend. The arrows show the elution positions of the two diastereomers of 8,5'-cyclo-dGuo. The full scales of the m/z -464 and m/z -524 ions are 4 times those of the other ions shown here.

Fig. 7. Mass spectrum generated on the basis of the ions and their area counts at the elution positions shown with the arrows in Fig. 6

with a flame ionization detector. (*S*)-8,5'-Cyclo-Ado was used as an internal standard. The relative molar response factors of the trimethylsilyl derivatives of these compounds for the flame ionization detector were assumed to be equal because of the same functional groups carrying trimethylsilyl groups. The *G* value (number of molecules formed per 100 eV of absorbed radiation energy) of 8,5'-cyclo-dGuo amounted to 0.15 and 0.6 for N_2O -saturated DNA and dGuo samples respectively. In N_2 -saturated solutions these values were 0.06 and 0.2 respectively. The presence of O_2 in solutions of dGuo and DNA during γ -irradiation inhibited the formation of 8,5'-cyclo-dGuo.

DISCUSSION

$\cdot OH$ radicals generated by γ -irradiation in aqueous solution react with both the heterocyclic bases and the sugar moiety of DNA. Predominant reactions are the addition of $\cdot OH$ radicals to the double bonds of the bases. To a lesser extent, the abstraction of hydrogen atoms from the carbon atoms of the sugar moiety also occurs (for a review see Scholes, 1978). Reactions of the resulting sugar radicals lead to strand breaks and/or base release with the formation of sugar products (Dizdaroglu *et al.*, 1975; Beesk *et al.*, 1979).

The formation of the 8,5'-cyclo-dGuo moiety in DNA and dGuo can be explained by analogy to the $\cdot OH$ -induced formation of 8,5'-cyclo-AMP from AMP or of 8,5'-cyclo-dAdo from dAdo (Keck, 1968; Raleigh *et al.*, 1976; Mariaggi *et al.*, 1976) by hydrogen-atom abstraction by $\cdot OH$ radicals from the C-5' of the sugar moiety. The attack of the resulting sugar radical at the C-8=N-7 double bond of the guanine ring would lead to intramolecular cyclization with the formation of an N-7-centred radical. Oxidation of this radical might result in formation of the 8,5'-cyclo-dGuo moiety. The absence of this product in the presence of O_2 supports this mechanism because the well-known diffusion-

controlled reaction of O_2 with free radicals would prevent intramolecular cyclization reaction of the C-5'-centred sugar radical. The increased yield of this product in N_2O -saturated solutions underlines the role of $\cdot OH$ radicals in its formation.

Previous papers reported that $\cdot OH$ -induced intramolecular cyclization of dAdo is highly stereoselective and generates only the (*R*)-epimer of 8,5'-cyclo-dAdo (Mariaggi *et al.*, 1976; Cadet & Berger, 1985). On the other hand, Raleigh & Fuciarelli (1985) showed the formation of both (*R*)- and (*S*)-epimers of 8,5'-cyclo-AMP from AMP. The results presented here also clearly demonstrate the formation of both epimers of 8,5'-cyclo-dGuo in dGuo as well as in DNA. The measured ratio of the (*R*)-epimer to the (*S*)-epimer was approx. 5:1 in dGuo or 3:1 in DNA.

The formation of an 8,5'-cyclo-dGuo moiety in DNA might be quite interesting from the viewpoint of DNA repair. This lesion presents a simultaneous damage to the base and sugar moieties of the same nucleotide subunit and, also, may cause a conformational distortion of the secondary structure of DNA. Its repair by repair enzymes (if repaired at all) might therefore be different from that of other base lesions with an intact sugar moiety. DNA glycosylases, which excise some forms of base damage as the free base by catalysing the hydrolysis of the *N*-glycosidic bonds, probably would not be able to act on such a lesion because of the C-8-C-5' covalent bond. On the other hand, endonucleases that recognize conformational distortions of the secondary structure of DNA (Friedberg, 1984) might be active in the repair of 8,5'-cyclo-dGuo. Moreover, repair enzymes might act differently on the (*R*)- and (*S*)-epimers of this compound.

In that respect, the release of 8,5'-cyclo-dGuo moieties from DNA by exonucleases is also noteworthy (see the Results section). A previous work (Dizdaroglu *et al.*, 1978) has shown that γ -irradiated DNA was not completely hydrolysable by mixtures of endo- and

exo-nucleases. Some oligonucleotides remained that were resistant to enzymic hydrolysis. However, lesions causing this resistance have not been elucidated. The fact that 8,5'-cyclo-dGuo was present in enzymic hydrolysates of γ -irradiated DNA as revealed here indicates the ability of spleen and snake-venom exonucleases to hydrolyse oligonucleotides containing this residue.

CONCLUSIONS

The reactions of ionizing radiation-generated $\cdot\text{OH}$ radicals with dGuo and DNA in aqueous solutions in the absence of O_2 have been shown to lead to the formation of an 8,5'-cyclo-dGuo moiety. Both (*R*)- and (*S*)-epimers were observed. This product could be detected in γ -irradiated DNA by the use of the g.c.-m.s./s.i.m. technique at radiation doses as low as 1 Gy. Its formation is believed to result from an initial abstraction of a hydrogen atom from the C-5' atom of the sugar moiety followed by intramolecular cyclization between C-5' and C-8 and subsequent oxidation of the resulting N-7-centred radical.

The repair of this lesion in DNA might be different from that of other base lesions with an intact sugar moiety and involve repair enzymes that recognize conformational distortions of DNA rather than particular forms of base damage.

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