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INDUCTION OF DNA STRAND BREAKS BY RSU-1069, A NITROIMIDAZOLE-AZIRIDINE RADIOSENSITIZER

ROLE OF BINDING OF BOTH UNREDUCED AND RADIATION-REDUCED FORMS TO DNA, *IN VITRO*

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Abstract—[2-^{14}C]-RSU-1069 [1-(2-nitro-1-imidazolyl)-3-(1-aziridino)-2-propanol], either as a parent (unreduced) or following radiation reduction, binds to calf thymus DNA *in vitro*. Radiation-reduced RSU-1069 binds to a greater extent and more rapidly than the parent compound. RSU-1137, a non-aziridino analogue of RSU-1069, binds following radiation reduction. Radiation-reduced misonidazole (1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol) exhibits binding ratios a thousand-fold less than those of reduced RSU-1069. There is no evidence for binding of parent misonidazole. Both parent and reduced RSU-1069 cause single strand breaks (ssbs) in pSV2 gpt plasmid DNA with the reduced compound causing a greater number of breaks. Parent and reduced RSU-1137 and misonidazole do not cause ssbs. It is inferred that the aziridine moiety present in both parent and reduced RSU-1069 is required for ssb production. RSU-1069 reacts with inorganic phosphate probably via nucleophilic ring-opening of the aziridine fragment. Incubation of plasmid DNA with reduced RSU-1069 in the presence of either phosphate or deoxyribose-5-phosphate at concentrations greater than 0.35 mol dm^{-3} prevents strand breakage, whereas 1.2 mol dm^{-3} deoxyribose does not protect against strand breakage formation.

From these findings it is proposed that the observed binding to DNA occurs via the aziridine and the reduced nitro group of RSU-1069 and that these two have different target sites. Binding to DNA via the reduced nitro group may serve to increase aziridine attack due to localization at or near its target.

RSU-1069 [1-(2-nitro-1-imidazolyl)-3-(1-aziridino)-2-propanol] is a more efficient hypoxic radiosensitizer and chemopotentiator than misonidazole (1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol) both *in vitro* and *in vivo* [1–2]. Both compounds show marked cytotoxicity towards hypoxic cells *in vitro*, with RSU-1069 being more toxic at the same concentration. RSU-1069 is also highly cytotoxic towards oxic cells, an effect only shown by misonidazole after long incubation times. These results may reflect differences in the mechanism of action of the two compounds since RSU-1069 differs chemically from misonidazole in the *N*-1 side-chain (structures in Fig. 1).

Chemically-reduced [2-^{14}C]misonidazole has been shown to bind *in vitro* to both calf thymus DNA and bovine albumin [3]. Varghese and Whitmore [3] suggested that, of the reduced products formed, the hydroxylamine derivative of misonidazole may be responsible for the binding seen in their *in vitro* systems. Parent (unreduced) misonidazole, on the other hand, did not bind to either protein or DNA.

DNA has been implicated as a target for the radiosensitization and cytotoxicity caused by RSU-1069 in mammalian cell systems (I.J. Stratford, personal communication). RSU-1069 has previously been shown to damage DNA by causing helix disruption leading to single strand breaks and thymine release [4]. The present study was undertaken to investigate the capacity of unreduced and radiation-reduced [2-^{14}C]-RSU-1069 and [2-^{14}C]-misonidazole (see Fig. 1 for structures) to bind to DNA *in vitro*. The binding of RSU-1137 (which is formed in aqueous solution by hydrolysis of the aziridine ring of RSU-1069) was also investigated. Plasmid (pSV2 gpt) DNA [5] was used to study the capacity of these compounds, in both reduced and unreduced states, to cause strand breakage. From these results mechanisms of interaction are presented.

MATERIALS AND METHODS

Preparation of RSU-1069 and [2-^{14}C]-RSU-1069. RSU-1069 was prepared by the procedure of Adams

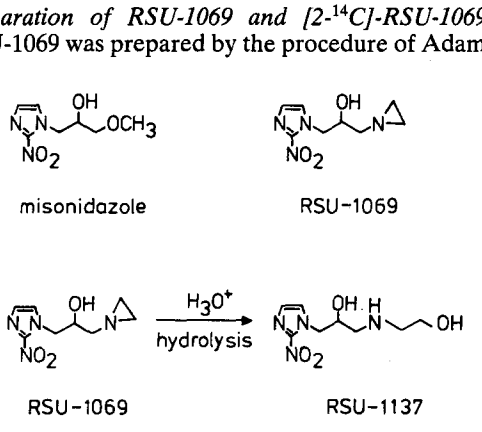

Fig. 1. Structures of misonidazole, RSU-1069 and RSU-1137.

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et al. [1] and had melting point, spectroscopic parameters and chromatographic behaviour identical with that of authentic material. [2-^{14}C]-labelled RSU-1069 was prepared by conversion of a mixture of 2-nitroimidazole (480 mg) and [2-^{14}C]-2-nitroimidazole (NSC-105831, 20 mg, 629 MBq mmol^{-1}) to the 1-(2,3-epoxypropyl) derivative using the two-stage procedure of Beaman *et al.* [6]. The epoxide (447 mg) was then refluxed with methanol (4 cm^3) and aziridine (0.6 cm^3) for 45 min, filtered and concentrated *in vacuo*. Treatment with diethyl ether and crystallization from ethanol-ether gave [2-^{14}C]-RSU-1069 as pale-yellow prisms (354 mg), mp 390–391.5 K (lit.: 392–394 K [1]), with specific activity 25.2 MBq mmol^{-1}. This compound was homogenous by thin-layer chromatography and hplc and indistinguishable from the unlabelled material by u.v. and i.r. spectrophotometry.

Preparation of [2-^{14}C]-RSU-1137. [2-^{14}C]-Labelled RSU-1137 (specific activity 25.2 MBq mmol^{-1}) was prepared from [2-^{14}C]-RSU-1069 by incubation in perchloric acid solution, pH 2.0. for 48 hr at 37°. The solution was then neutralized with sodium hydroxide, assayed using HPLC and the concentration determined using a Beckman DU-8B spectrophotometer. The percentage hydrolytic conversion to RSU-1137 was > 97%.

[2-^{14}C]-misonidazole. [2-^{14}C]-Misonidazole with a specific activity of 393.8 MBq mmol^{-1} was a gift from Dr. C. E. Smithen, Roche Products Ltd. (Welwyn Garden City, U.K.).

Radiation reduction of RSU-1069, RSU-1137 and misonidazole. The 4.3 MeV linear accelerator and the optical detection and analysis systems used in the radiation studies of RSU-1069 and preparation of solutions have previously been described [7]. In order to investigate the properties of the one-electron reduced species of RSU-1069, solutions prior to irradiation were saturated with N_2O in the presence of 0.1 mol dm^{-3} formate and 2 mmol dm^{-3} phosphate at 295 ± 3 K. In this system e^-_{aq} is converted by N_2O into $^{\cdot}OH$ radicals which, on interaction with formate (Scheme 1), produce the reducing radical, $CO_2^{\cdot-}$. As shown in Scheme 2, the one-electron reduced species of RSU-1069 ($ArNO_2^{\cdot-}$) is produced on interaction of $CO_2^{\cdot-}$ with the nitroarene ($ArNO_2$).

$$^{\cdot}OH\,(H^{\cdot}) + HCO_2^- \rightarrow H_2O(H_2) + CO_2^{\cdot-}$$

(Scheme 1)

$$CO_2^{\cdot-} + ArNO_2 \rightarrow CO_2 + ArNO_2^{\cdot-}$$

(Scheme 2)

Radiation doses (10–30 Gy pulse^{-1}) were determined using KSCN dosimetry at 480 nm assuming G = 0.3 μmol J^{-1} and ε = 710 m^2 mol^{-1}.

In order to investigate the interaction of reduced products of RSU-1069, RSU-1137 and misonidazole with DNA, N_2— saturated aqueous solutions of the nitroarenes containing 0.1 mol dm^{-3} HCO_2^- and buffered at pH 7.0 ± 0.2 with 40 mmol dm^{-3} phosphate were irradiated with a train of electron pulses generated by the linear accelerator at a rate of approximately 20 pulses min^{-1}. This concentration of phosphate was sufficient to maintain the pH of the solutions which were bubbled with N_2 during the period of irradiation. The dose/pulse of 43 Gy was determined using a modified Fricke dosimeter saturated with O_2. Under these conditions and based upon a G (reducing equivalents) of 0.65 μmol J^{-1}, the concentration of reducing equivalents produced per pulse is approximately 25.8 μmol dm^{-3}.

Optical absorption spectra of both unirradiated and irradiated solutions were recovered using a Beckman DU-8B spectrophotometer so that the concentration of remaining parent nitroarene could be determined. In the majority of experiments, the nitroimidazole was irradiated to produce ≥90% conversion of drug to reduced product(s).

Binding to calf thymus DNA. Calf thymus DNA (0.4 mg cm^{-3}) was dissolved in 40 mmol dm^{-3} phosphate buffer, pH 7.0, containing 0.1 mol dm^{-3} sodium formate and then stored at 277 K prior to use. Drugs (2 mmol dm^{-3} unless indicated otherwise) were prepared in the same buffer system. Binding studies were initiated by mixing equal volumes (normally 2.5 cm^3) of aerated DNA solution and either radiation-reduced or unreduced RSU-1069, RSU-1137 or misonidazole followed by incubation at 310 K. The time taken from initiation of pulsed irradiation to mixing with DNA was 15 min. At appropriate time intervals, 0.3 cm^3 aliquots were removed and added to 60 mm^3 of 2 mol dm^{-3} sodium acetate. After addition of ice-cold ethanol (0.9 cm^3) samples were spun at 12,000 *g* for 4 min, the supernatant then removed, and the pelleted DNA resuspended in 0.3 cm^3 of 20 mmol dm^{-3} sodium cacodylate buffer, pH 7.0. The DNA was then reprecipitated using 60 mm^3 of 2 mol dm^{-3} sodium acetate/0.9 cm^3 of ice-cold ethanol and washed onto a 2.1 cm Whatman GF/C filter paper with ice-cold ethanol. The filter was washed with 10 cm^3 of ice-cold ethanol, allowed to dry at room temperature for 20 min and placed in a glass scintillation vial containing 10 cm^3 scintillant (scintillator 299 TM from United Technologies Packard). After counting in a Packard Tricarb Liquid Scintillation spectrometer the counts were corrected for background by establishing the amount of ^{14}C bound at zero time. For this, the DNA was precipitated immediately after mixing with labelled drug and prepared for counting as above. Background accounted for approximately 0.2% of the total count in each 0.3 cm^3 aliquot. Binding ratios are expressed in terms of the number of molecules of drug bound per 100 nucleotides.

Determination of strand breaks on pSV2 gpt plasmid. The plasmid pSV2 gpt was maintained in *E. coli* HB101 and plasmid DNA extracted using conventional methods [8]. DNA concentrations were determined spectrophotometrically. Plasmid, stored at a concentration of 1 mg cm^{-3} in 10 mmol dm^{-3} Tris–HCl and 2 mmol dm^{-3} EDTA, pH 8.0, was prepared for exposure to drugs by addition of 50 mm^3 plasmid stock solution to 236 mm^3 of 20 mmol dm^{-3} sodium cacodylate buffer, pH 7.0. Parent or reduced drug was prepared as detailed above and then mixed (25 mm^3) with 10 mm^3 plasmid DNA and incubated at 310 K. Final plasmid concentration was approximately 50 μg cm^3.

The ability of phosphate, deoxyribose-5-phos-

phate and deoxyribose to protect the plasmid was tested by preparing solutions of reduced or parent RSU-1069 as detailed above but at a five-fold greater concentration. The deoxyribose-5-phosphate was prepared in 40 mmol dm^{-3} phosphate buffer, pH 7.0. RSU-1069 was diluted with the potential protectant to give a final drug concentration of 0.3 mmol dm^{-3} and a known concentration of protectant in a solution of pH 7.0. The resulting RSU-1069 solution was then immediately added to plasmid DNA and assayed for strand breakage following incubation for 3 hr at 310 K.

Single strand breaks (ssbs) were assayed by agarose (0.8%) gel electrophoresis (Tris–borate/EDTA buffer . (178 mmol dm^{-3}/5 mmol dm^{-3}) + 1 μg ethidium bromide) at 70 V for 45 min. Gels were excited with a u.v. transilluminator and photographed using a Polaroid camera equipped with a orange/red filter and Type 55 Land films. The negative photographs were assayed for strand breakage by densitometry using a Beckman DU-8B spectrophotometer fitted with a gel scanner accessory.

Densitometry data were expressed two ways; (i) the darkened area of the negative associated with the open-circular form (type II) of plasmid DNA treated with drugs was expressed as a percentage of that area associated with the open-circular form of the untreated plasmid DNA, or (ii) the areas of open (type II)- and closed (type I)-circular DNA was expressed as a ratio (open-circular : closed-circular).

Stability of RSU-1069 in phosphate and acetate buffers. RSU-1069 (20 mmol dm^{-3}) in either sodium acetate or phosphate buffer solution (50 mmol dm^{-3}, pH 7.0) was incubated at 310 K. Aliquots were assayed for drug stability at hourly intervals using a Beckman 344 HPLC system (5 μm Spherisorb-CN; 10% methanol in 10 mmol dm^{-3} KH_2PO_4, pH 3.0, as mobile phase; 310 nm).

RESULTS

Properties of one-electron reduced RSU-1069

The optical absorption spectrum, 29 μsec after pulse-irradiation of a N_2O-saturated solution of RSU-1069 (456 μmol dm^{-3}) at pH 7.0, is similar to that determined for other one-electron reduced 2-nitroimidazoles [9]. The decay of one-electron reduced RSU-1069 is second-order for least 2.5 half-lives in the dose/pulse range > 30 Gy. From the dependence of the rate of change of optical absorption with time at 420 nm, the second-order rate constant was found to be $2k_2 = 1.0 (\pm 0.2) \times 10^6$ dm^3 mol^{-1} sec^{-1} at pH 7.1 using the determined ε of 105 m^2 mol^{-1} at 420 nm. At a dose/pulse of < 10 Gy, the rate of decay of the one-electron reduced species of RSU-1069, as determined at 420 nm, is approximately first-order with k_1 about 5.7 sec^{-1}. The kinetics of decay were not studied further but were consistent with the apparent dose-dependent, first-order rate of decay [10], for the one-electron reduced misonidazole species. The reasons for the differences in the kinetic behaviour are not known. The optical absorption spectra of the one-electron reduced species of RSU-1069 were also determined at pH 5.8, 7.9 and 10.9. Only at pH 5.8 was a slight shift of the absorption spectra apparent; from these observations it is inferred that the pK_a of the one-electron reduced species of RSU-1069 is < 6.0.

The rate constant for interaction of oxygen with the one-electron reduced species of RSU-1069 was determined to be 3.6×10^6 dm^3 mol^{-1} sec^{-1} from the dependence of the first-order rate of decay at 420 nm of the one-electron reduced species of RSU-1069 on O_2 (86–260 μmol dm^{-3}).

Stoichiometry of reduction

On irradiation with a train of electron pulses, of an aqueous solution of RSU-1069 at pH 7.1 the optical absorption of RSU-1069 with a peak at 325 nm decreases with dose and there is no observable formation of a product which absorbed in the 220–450 nm wavelength. The loss of optical absorption at 325 nm of RSU-1069 (700 μmol dm^{-3}) was found to be linearly dependent upon the dose delivered even up to conversions of approximately 90%. Assuming a total yield for reducing species of 0.65 μmol J^{-1} [11], 3.7 ± 0.3 reducing equivalents are accepted per RSU-1069 molecule reduced.

Stability of RSU-1069 in phosphate and acetate buffers

As shown in Fig. 2 the stability of RSU-1069 is less in phosphate than in sodium acetate solution. HPLC analysis of the phosphate solution revealed formation of a new major component eluting before both RSU-1069 and the hydrolysis product RSU-1137 (peaks A, C and B respectively in Fig. 2 (inset)).

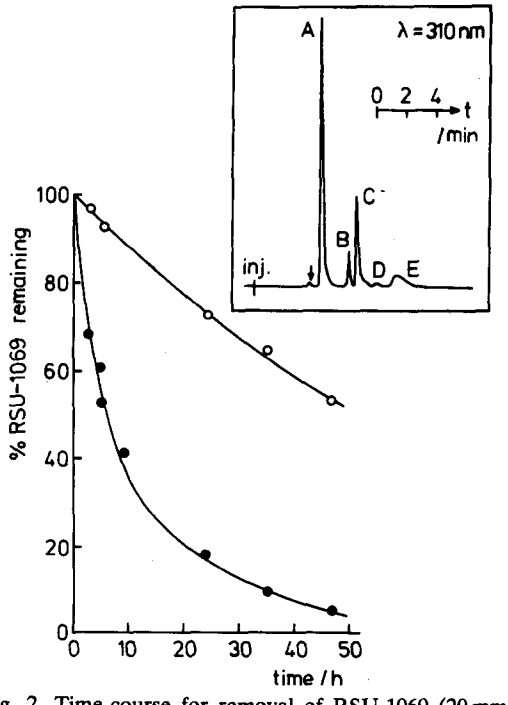

Fig. 2. Time-course for removal of RSU-1069 (20 mmol dm^{-3}) in buffered, aqueous solution containing sodium acetate (○, 50 mmol dm^{-3}) or phosphate (●, 50 mmol dm^{-3}) at 310 K, pH 7.0. Inset: HPLC chromatogram (conditions given in text) of RSU-1069 incubated in phosphate buffer: peak A (0.9 absorbance unit) represents the major ionic product; B = RSU-1137; C = RSU-1069; D and E = minor products.

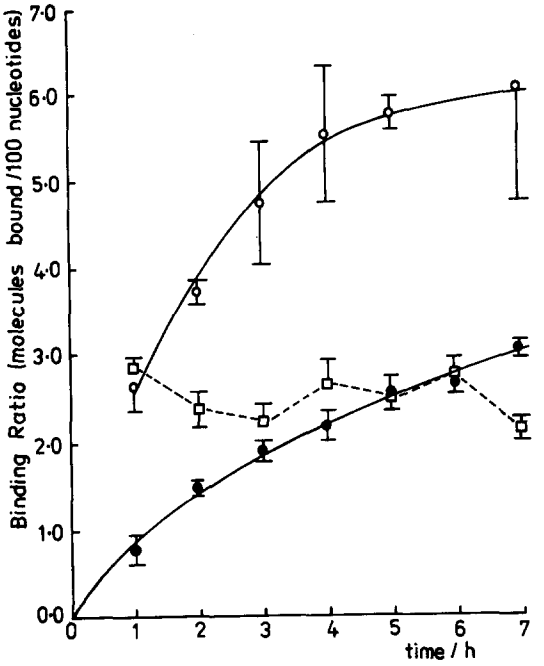

Fig. 3. Time dependence of binding to calf thymus DNA of unreduced (●) and radiation-reduced (○) RSU-1069 and of radiation-reduced RSU-1137 (□). Points are means from at least 3 determinations ± S.E.M. (vertical bars).

Two minor (< 5%) products of RSU-1069 degradation (peaks D and E) are also evident. Preliminary studies indicate that component A is an ionic salt product resulting from nucleophilic attack upon the RSU-1069 aziridine ring by phosphate anion (Silver, O'Neill and Jenkins, unpublished data, 1985). Solutions of RSU-1069 in acetate buffer do not produce peaks A, D or E.

Binding of RSU-1069, RSU-1137 and misonidazole to calf thymus DNA

Radiation-reduced RSU-1069 binds to a greater extent and more rapidly than the parent compound as shown in Fig. 3; for example, the binding ratio for reduced drug is approximately 3.5 times that of the parent RSU-1069 within 1 hr (Fig. 3). On the other hand, unreduced RSU-1137 in the parent form does not bind to DNA whereas the reduced drug binds rapidly within the first hour with no subsequent binding (Fig. 3). The level of binding is equivalent to that determined using reduced RSU-1069 after 1 hr.

Both the radiation-reduced and parent forms of misonidazole (1 mmol dm^{-3}) do not bind significantly; binding ratios were determined as $< 0.04 \pm 0.01$ after 5 hr incubation at 310 K. At the higher concentration of 2.5 mmol dm^{-3}, reduced misonidazole gives a binding ratio of 0.22 ± 0.01 (3 reps.) after 5 hr incubation with calf thymus DNA, whereas, reduced RSU-1069 (1 mmol dm^{-3}) recorded a binding ratio of 6.12 ± 0.15 (3 reps.) after only 1 hr incubation.

Single-strand breaks in pSV2 qpt plasmid

Both radiation-reduced and parent RSU-1069 were found to induce single strand breaks (ssbs) in pSV2 gpt plasmid DNA. Following treatment with RSU-1069 the closed-circular form of DNA is removed with a resulting increase of the open-circular form and, at longer times, the linear form (Type III) appears with associated loss of the open-circular form of DNA. The DNA is eventually degraded by the RSU-1069, as indicated by the absence of distinct bands on the gel.

The quantity of open-circular DNA present during 5 hr exposure at 310 K to 0.3 mmol dm^{-3} parent or reduced RSU-1069 was established. The closed-circular DNA is converted to the open-circular form more rapidly by irradiated than unirradiated RSU-1069 (Fig. 4). Net loss of the open-circular form also occurs after a shorter incubation time with reduced (after 2 hr) than parent (after 4 hr) RSU-1069 (Fig. 4). Complete degradation of plasmid DNA is achieved after 3 hr incubation with irradiated RSU-1069; parent RSU-1069 causes the DNA to smear only after incubation > 5 hr.

Parent and reduced forms of RSU-1137 (0.3 mmol dm^{-3}) and misonidazole (1.3 mmol dm^{-3}) do not cause any strand breaks to the plasmid DNA even after 5 and 24 hr incubation respectively. Furthermore, RSU-1137 and misonidazole do not alter the ratio of open- to closed-circular DNA from that determined for the controls.

Incubation of plasmid with reduced RSU-1069 in the presence of 0.35 mol dm^{-3} phosphate or 0.35 mol dm^{-3} 2-deoxyribose-5-phosphate (Fig. 5) prevents strand breakage whereas the presence of 1.2 mol dm^{-3} 2-deoxyribose does not modify strand breakage yields. Total loss of the closed-circular form of DNA occurs using phosphate or ribose-phosphate at concentrations $\leqslant 0.07$ mol dm^{-3}.

DISCUSSION

The properties determined for the one-electron

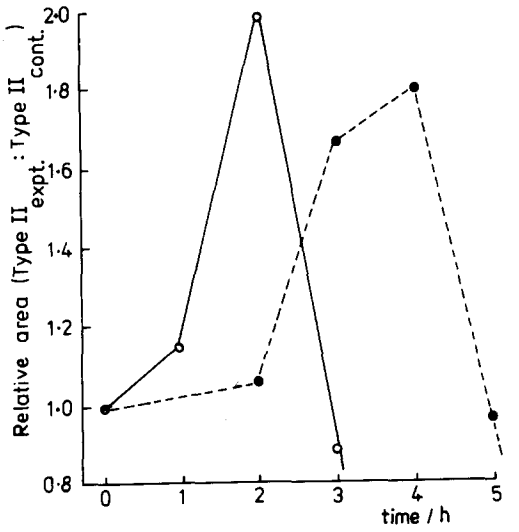

Fig. 4. The quantity of open-circular (type II) plasmid DNA (pSV2 gpt) present over a period of exposure to unreduced (●) or radiation-reduced (○) RSU-1069. Data is expressed relative to control plasmid not exposed to drug.

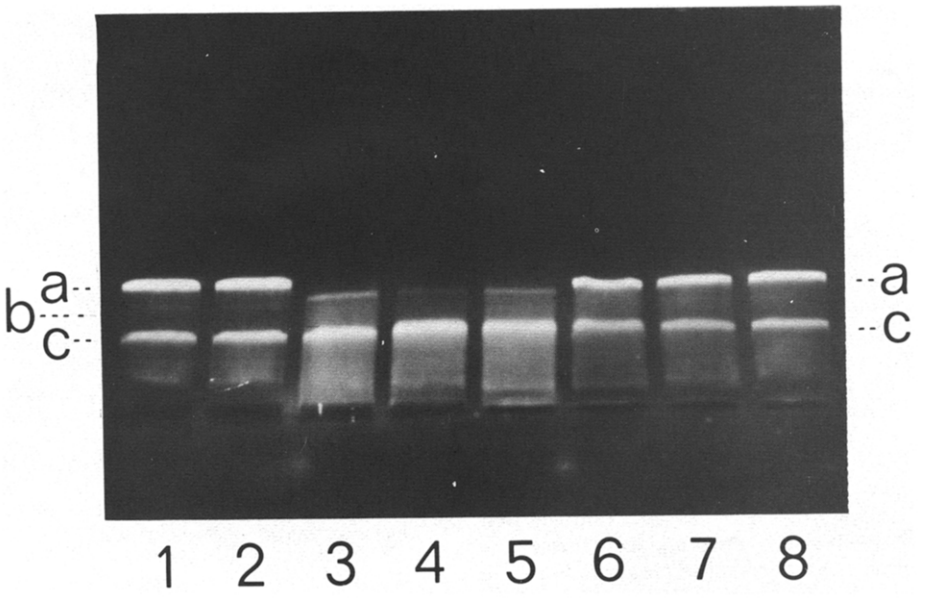

Fig. 5. Protection of plasmid DNA (pSV2 gpt) from strand breakage caused by radiation-reduced RSU-1069 using 2-deoxyribose and 2-deoxyribose-5-phosphate: (a) closed circular; (b) linear; (c) open circular DNA. Lanes 1 and 2 = untreated, control, plasmid; lanes 3–5 show effects of 1.2, 0.6 and 0.3 mol dm^{-3} 2-deoxyribose, respectively; lanes 6–8 show protection by the same concentrations of 2-deoxyribose-5-phosphate.

reduced species of RSU-1069 are similar to those determined for the one-electron reduced species of misonidazole [9–12]. Furthermore, the stoichiometry of RSU-1069 reduction is consistent with previous determinations for the 2-nitroimidazoles RSU-1069 [4] and misonidazole [11, 14–20] but is in contrast with the finding of Raleigh and Liu [13] whereby only 3 reducing equivalents per misonidazole molecule were accepted. At present, the reduction products of 2-nitroimidazoles are not fully characterized, although the 2-hydroxylamine has been proposed as the initial and major product following chemical reduction of misonidazole [3, 21, 22]. In this discussion it is proposed that a non-specified product(s) formed by radiation reduction of the nitroimidazole moiety of RSU-1069 and RSU-1137 binds to DNA *in vitro*. The non-specified product(s) which binds to DNA must result in the incorporation of the ^{14}C label. We do not observe similar binding with radiation-reduced [2-^{14}C]-misonidazole. Based upon the observation of McClelland *et al.* [21] concerning the lifetime of the hydroxylamine of misonidazole at pH 7, it is assumed that the binding witnessed with the radiation-reduced RSU-1069 probably does not involve the hydroxylamine product directly. In contrast, a ^{14}C-containing product of chemically-reduced [2-^{14}C]-misonidazole has been found to bind to DNA [3]. Furthermore, Varghese and Whitmore [22] report the binding of a glyoxal fragment, formed from the hydroxylamine derivative of chemically-reduced misonidazole, to the free base guanosine, a process which does not result in the incorporation of the ^{14}C label. Hence, the absence of binding of radiation-reduced [2-^{14}C]misonidazole to DNA in our experiments does not rule out the possibility of binding via glyoxal or other non-[2-^{14}C] fragments. It should also be stressed that our experiments involved addition of 2-nitroimidazole to DNA under aerobic conditions, following radiation reduction of the test compound. The reduced drugs were incubated with DNA in an aerobic environment.

The observed binding of radiation-reduced RSU-1137, but not radiation reduced [2-^{14}C]-misonidazole, may indicate the importance of the *N*-1 side chain in effecting the binding of [2-^{14}C]-imidazole fragments into DNA. The nature of the *N*-1 side chain and, in particular, the presence of a positively-charged nitrogen function, may be of relevance. A charge at this position may lead to localization and/or electrostatic attraction of the reduced metabolite(s) to the DNA target site. This possibility is being investigated using tertiary amine and quaternary salt analogues related to RSU-1069.

[2-^{14}C]-RSU-1069, either as parent (unreduced) or following radiolytic reduction, has been shown to bind to calf thymus DNA *in vitro*. It is proposed that the binding of parent RSU-1069 involves the aziridine group since the non-aziridine 2-nitroimidazoles (RSU-1137 and misonidazole) used as parent compounds in this study do not bind. It has been demonstrated that a consequence of aziridine binding to DNA is strand breakage as evidenced by the fact that 1-aziridineethanol (Silver, O'Neill and Jenkins, unpublished data, 1985) is as efficient as RSU-1069 in causing strand breakage.

From the observed instability of the aziridine group in the presence of inorganic phosphate and from the observed protection against strand breakage by phosphate and deoxyribose-5-phosphate, it is suggested that the phosphate of DNA is one potential target for attack by the aziridine of RSU-1069. Indeed, alkylation of phosphates by the aziridine

group of mitomycin C has previously been shown to occur [23].

From the inability of radiation-reduced RSU-1137 to induce ssbs in plasmid DNA but still bind to DNA, it is proposed that an alternative site for binding to that of the aziridine function exists for the reduced nitroimidazole fragment(s) of RSU-1069 and RSU-1137.

As seen from the timescale of strand break formation, radiation-reduced RSU-1069 degrades plasmid DNA more readily than an equivalent concentration of the unreduced compound. The timescale over which the reduced nitroimidazole fragment binds to DNA may be of importance in explaining this phenomenon. For example, under the conditions used RSU-1137 binds rapidly over the first hour with no further binding taking place thereafter (Fig. 3). RSU-1069, on the other hand, continues to bind at a significant rate after 1 hr. If it is assumed, using RSU-1137 as an example, that binding via the reduced nitroimidazole moiety is complete within 1 hr, then subsequent binding with reduced RSU-1069 may occur via the aziridine of a product(s) of radiation reduction and/or unreduced RSU-1069 (since the reduction is carried out to $\geq 90\%$ completion).

In conclusion, radiation-reduced RSU-1069 may cause an increased rate of ssb production if binding initially occurs via a product of nitro-reduction followed by an intramolecular interaction with the aziridine group. Initial binding could result in localization of the aziridine at or near the sugar-phosphate backbone of DNA. It is therefore inferred that radiation reduced RSU-1069 may also act as a *bifunctional* alkylating agent whereas radiation-reduced RSU-1137 and parent RSU-1069 act only as monofunctional agents. These findings contrast with those of Edwards *et al.* [4] who suggested that electrochemically-reduced and parent RSU-1069 only behave as monofunctional agents with respect to DNA ssbs.

The bifunctional alkylating character of reduced RSU-1069 probably plays a role in both the enhanced radio-sensitization and increased anaerobic cytotoxicity of RSU-1069 (I. J. Stratford, personal communication) whereas, under aerobic conditions, the aziridine function may alone account for the observed toxicity. The capacity of RSU-1069 to act as a mono- or bifunctional alkylating agent will be critically dependent upon cellular oxygen tension or the presence of other bioreductive drugs.

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