values used in eqs A18 and A22.

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DNA Cross-Linking and Sequence Selectivity of Aziridinylbenzoquinones: A Unique Reaction at 5′-GC-3′ Sequences with 2,5-Diaziridinyl-1,4-benzoquinone upon Reduction†

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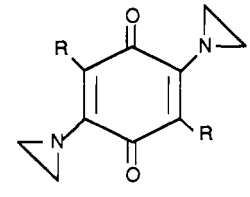
ABSTRACT: Several bifunctional alkylating agents of the aziridinylbenzoquinone class have been evaluated as potential antitumor agents. 3,6-Bis[(2-hydroxyethyl)amino]-2,5-diaziridinyl-1,4-benzoquinone (BZQ), 2,5-diaziridinyl-1,4-benzoquinone (DZQ), 3,6-bis(carboxyamino)-2,5-diaziridinyl-1,4-benzoquinone (AZQ), and six analogues of AZQ have been studied for their ability to induce DNA interstrand cross-linking, as measured by an agarose gel technique, and to determine whether they react with DNA in a sequence-selective manner, as determined by a modified DNA sequencing technique. At an equimolar concentration (10 μM), only DZQ and BZQ showed any detectable cross-linking at pH 7 without reduction. Cross-linking was enhanced in both cases at low pH (4). Reduction by ascorbic acid at both pH's increased the cross-linking, which was particularly striking in the case of DZQ. In contrast, AZQ and its analogues only produced a significant level of cross-linking under both low-pH and reducing conditions, the extent of cross-linking decreasing as the size of the alkyl end group increased. The compounds reacted with all guanine-N7 positions in DNA with a sequence selectivity similar to other chemotherapeutic alkylating agents, such as the nitrogen mustards, although some small differences were observed with BZQ. Nonreduced DZQ showed a qualitatively similar pattern of reactivity to the other compounds, but on reduction (at pH 4 or 7) was found to react almost exclusively with 5′-GC-3′ sequences, and in particular, at 5′-TGC-3′ sites. A model to explain this unique reaction is proposed.

Several natural and synthetic quinones including adriamycin, mitomycin C, and aziridinylbenzoquinones have found an application as antitumor agents. Such compounds have the potential to undergo reduction by cellular enzymes to produce more active forms (Butler & Hoey, 1987; Powis, 1987) although it has been difficult to prove the role of bioreductive activation in the antitumor activity or the production of toxic side effects of these quinones, and a single mechanism cannot fully explain all the observed cytotoxic effects.

Two aziridinylbenzoquinones, AZQ (diaziquone, Figure 1, compound D2) and BZQ (Figure 1), have undergone clinical trials as potential antitumor drugs (Khan & Driscoll, 1979; Bender et al., 1983; Haid et al., 1985). Aziridines have the potential to react with available nucleophiles to form ring-opened covalent adducts. Many aziridinylbenzoquinones have the added advantage in that the ring-opening process would



COMPOUND	R
BZQ	$NHCH_2CH_2OH$
DZQ	H
D1	$NHCOOCH_3$
D2 (AZQ)	$NHCOOC_2H_5$
D3	$NHCOOC_3H_7$ (*n*-propyl)
D4	$NHCOOC_3H_7$ (*i*-propyl)
D5	$NHCOOC_4H_9$ (*n*-butyl)
D6	$NHCOOC_4H_9$ (*i*-butyl)
D7	$NHCOOC_4H_9$ (*sec*-butyl)

FIGURE 1: Structures of the aziridinylbenzoquinones used in this study.

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be facilitated by the change of electron distribution when the nonaromatic quinone is reduced to an aromatic semiquinone or hydroquinone (Butler et al., 1988; Lusthof et al., 1989). Both AZQ and BZQ alkylate and cross-link DNA (Szmigiero & Kohn, 1984; Szmigiero et al., 1984; Butler et al., 1989), but the cytotoxic mechanisms of the two compounds clearly differ (Butler et al., 1990). Whereas AZQ can readily be reduced to semiquinone radicals in a biological system under normal physiological conditions (Butler et al., 1988), BZQ is not easily reduced, and it was concluded that this agent functions as a bifunctional alkylating agent by an acid-catalyzed aziridine ring-opening mechanism and that other factors, including oxidation or reduction, are much less important (Butler et al., 1989).

It is therefore clear that small changes in the structure of aziridinylbenzoquinones can lead to different chemical and cytotoxic mechanisms. In the present study nine structurally related compounds (Figure 1) have been studied for their ability to induce DNA interstrand cross-linking under differing reducing and pH conditions. In addition, the influence of the ring substituents on the alkylation of DNA is examined by measuring the sequence selectivity of alkylation of guanine-N7 positions within a DNA sequence.

Materials and Methods

Chemicals. The aziridinylbenzoquinones BZQ, DZQ, and D1–D7 were synthesised according to previously described methods (Chou et al., 1976; Dzielendziak & Butler, 1989; Dzielendziak et al., 1990; Petersen et al., 1955). All compounds were checked for purity using melting point, ^{1}H NMR, IR, and mass spectral data. Initial quinone stock solutions were made up in DMSO at 10 mM. Electrophoresis-grade acrylamide and bis(acrylamide) were purchased from Sigma, ultrapure urea and agarose from BRL, and piperidine and ascorbic acid from BDH. [γ-^{32}P]ATP (5000 Ci/mmol) was from Amersham and pBR322 plasmid DNA from Northumbria Biologicals.

Enzymes. Restriction enzymes *Hin*dIII and *Eco*RI, T4 polynucleotide kinase (PNK), and bacterial alkaline phosphatase (BAP) were obtained from BRL.

Buffers. TEA is 25 mM triethanolamine and 1 mM EDTA, pH 7.2. TBE electrophoresis buffer is 90 mM Tris, 90 mM boric acid, and 2 mM EDTA, pH 8.3. Tris–acetate electrophoresis buffer is 40 mM Tris, 20 mM acetic acid, and 2 mM EDTA, pH 8.1. Alkylation stop solution is 0.6 M sodium acetate, 20 mM EDTA, and 100 μg/mL tRNA. Strand separation buffer is 30% dimethyl sulfoxide, 1 mM EDTA, 0.04% Bromophenol blue, and 0.04% xylene cylanol BAP buffer is 10 mM Tris-HCl and 120 mM NaCl, pH 8. PNK buffer is 60 mM Tris-HCl, 15 mM 2-mercaptoethanol, 10 mM $MgCl_2$, and 35 μM ATP, pH 7.8.

Preparation of End-Labeled DNA. pBR322 DNA was linearized by reaction with *Hin*dIII (2 units/μg, 37 °C, 1 h), dephosphorylated with BAP (3 units/μg, 65 °C, 1 h), and purified by standard phenol/chloroform extraction and ethanol precipitation reactions (Maniatis et al., 1982). The DNA was labeled at the 5′ ends with T4 PNK as described by Maxam and Gilbert (1980). For measurement of guanine-N7 alkylation the DNA was further cut with *Eco*RI (2 units/μg, 37 °C, 1 h).

Determination of DNA Interstrand Cross-Linking. The method has recently been described in detail (Hartley et al., 1991). Briefly, labeled DNA (~5000 cpm/sample) was incubated with drug in TEA buffer at 37 °C. Reactions were terminated by the addition of an equal volume of alkylation stop solution, and the DNA was immediately precipitated by the addition of 3 volumes of 95% ethanol. Following centrifugation and removal of supernatant, the DNA pellet was dried by lyophilization.

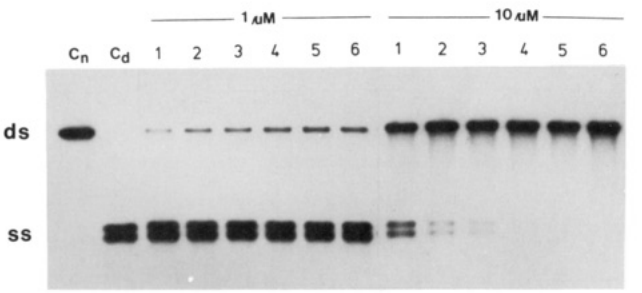

FIGURE 2: Autoradiogram of 0.8% neutral agarose gel showing the time course of interstrand cross-link formation in linear pBR322 DNA by BZQ, at pH 4, 37 °C, at either 1 or 10 μM. Times are (1) 20 min, (2) 40 min, (3) 60 min, (4) 90 min, (5) 120 min, and (6) 240 min. Lane C_n is control, nondenatured DNA, and lane C_d is denatured DNA in the absence of drug. ds and ss are double- and single-stranded DNA, respectively.

Samples were dissolved in 10 μL of strand separation buffer, heated at 90 °C for 2 min, and chilled immediately in an ice–water bath prior to loading. Control undenatured samples were dissolved in 10 μL of 6% sucrose and 0.04% Bromophenol blue and loaded directly. Electrophoresis was performed on 20-cm 0.08% submerged horizontal agarose gels at 40 V for 16 h with Tris–acetate running buffer.

Gels were dried at 80 °C onto filter paper, and autoradiography was performed at −70 °C. Quantitation was achieved by microdensitometry of the autoradiograph using a LKB Ultrascan-XL laser densitometer. For each lane the amount of single- and double-stranded DNA was determined and the percentage cross-linked (double-stranded) DNA calculated.

Determination of Sites of Guanine-N7 Alkylation. Singly end-labeled DNA (~50000 cpm/sample) was incubated with drug in TEA buffer in a total volume of 50 μL for 60 min at 20 °C. The reaction was terminated by the addition of 50 μL of cold alkylation stop solution and DNA recovered by precipitation with 3 volumes of 95% ethanol. The DNA was resuspended in 0.3 M sodium acetate and 1 mM EDTA and ethanol precipitated again, and the pellet was washed with cold ethanol prior to vacuum drying.

The salt-free DNA pellet was resuspended in freshly diluted 1 M piperidine and incubated at 90 °C for 15 min to convert quantitatively sites of guanine-N7 alkylation into strand breaks (Mattes et al., 1986a). Samples were lyophilized, resuspended in formamide loading buffer, heated at 90 °C for 1 min, and chilled in an ice bath prior to loading onto the gel. Electrophoresis was achieved in 0.4 mm × 80 cm × 20 cm 6% polyacrylamide gels containing 8 M urea. Running time was approximately 3 h at 3000 V, 55 °C. Gels were dried and autoradiographed, and relative band intensities were determined by microdensitometry as described above.

Molecular Modeling. Molecular modeling was carried out on a Silicon Graphics Iris 4D 70GT work station using QUANTA 3.0 software (including CHARMm 21.2) working under an IRIS O/S 3.3.2 operating system.

Results

DNA Interstrand Cross-Linking by Aziridinylbenzoquinones. DNA interstrand cross-linking was measured using a sensitive agarose gel based assay. A typical cross-link gel is shown in Figure 2 for BZQ at pH 4 and 37 °C. Quantitation is achieved by microdensitometry, and the resulting time course of percent cross-linked (double-stranded) DNA is shown in Figure 3, indicating a peak of cross-linking at 2 h. BZQ,

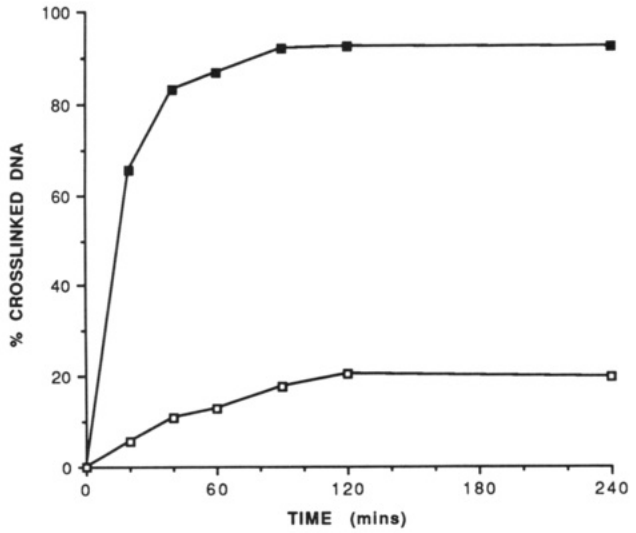

FIGURE 3: Time course of cross-linking for BZQ at 1 μM (open symbols) and 10 μM (closed symbols). Data are from gel shown in Figure 2.

Table I: Relative DNA Interstrand Cross-Linking by the Aziridinylbenzoquinones

	% double-stranded (cross-linked) DNA[b]			
compd[a]	pH 7	pH 7 + ascorbic acid	pH 4	pH 4 + ascorbic acid
BZQ	30	75	91	100
DZQ	8	100	69	100
D1	0	6	0.5	29.1
D2	0	4	0.4	21.6
D3	0	0	0	19.3
D4	0	0	1.1	22.2
D5	0	0	1.8	13.6
D6	0	0	0.8	14.6
D7	0	0	0.9	13.0

[a] Reactions at 10 μM drug were performed for 2 h at 37 °C. [b] Calculated from the amount of single- and double-stranded DNA determined from densitometry of agarose cross-link gel autoradiographs (see Materials and Methods).

DZQ, and compounds D1–D7 were compared using this assay at an equimolar concentration (10 μM) for 2 h under differing pH (4 vs 7) and reducing (±2 mM ascorbic acid) conditions. The results are presented in Table I. At this concentration BZQ produced cross-linking at pH 7 which was increased 3-fold when the pH was reduced to 4. Reducing conditions increased the extent of cross-linking at both pH values. DZQ was less efficient at producing DNA interstrand cross-linking than BZQ under nonreducing conditions, but cross-linking was considerably enhanced by reduction. In fact 100% cross-linked DNA was observed with DZQ at 1 μM, pH 4, + ascorbic acid (data not shown). Compounds D1–D7 did not show any cross-linking at pH 7 and less than 2% cross-linked DNA at pH 4. At pH 7 under reducing conditions only D1 and D2 showed any significant level of cross-linking, but all compounds gave cross-links under a combination of low-pH and reducing conditions. The extent of cross-linking under these conditions appeared to decrease as the size of the alkyl end group increased such that methyl (D1) > ethyl, propyl (D2–D4) > butyl (D5–D7).

Under the conditions used, ascorbic acid did not significantly alter the pH of the reaction mixture and similar results were obtained with other reducing agents such as sodium dithionite (data not shown).

Guanine-N7 Alkylation by Aziridinylbenzoquinones. The guanine-N7 position is the greatest site of reactivity on DNA by many chemotherapeutic alkylating agents. The DNA sequence selectivity for guanine-N7 alkylation by the aziridinylbenzoquinones was examined using a modified DNA sequencing technique. A portion of a typical gel autoradiograph is shown in Figure 4. The extent of overall guanine-N7 alkylation followed the same trend as the DNA interstrand cross-linking. BZQ and DZQ gave a comparable extent of alkylation at 10 μM, pH 4 (Figure 4, lanes e and c, respectively). This was enhanced a little upon reduction in the case of BZQ (lane f) and greatly enhanced in the case of DZQ, such that alkylation is clearly seen at 0.1 μM (lane d). With compounds D1–D7 a significant extent of alkylation was only observed under a combination of low-pH and reducing conditions at doses of 100 μM or above (e.g., compounds D1 and D2, Figure 4, lanes h and g, respectively).

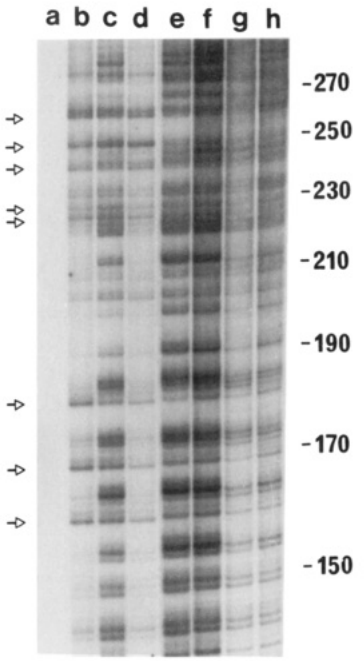

FIGURE 4: Portion of an autoradiogram of 6% denaturing polyacrylamide gel showing piperidine-induced DNA strand cleavage in a fragment of pBR322 DNA caused by guanine-N7 alkylation by aziridinylbenzoquinones. Lane a is control, unalkylated DNA. Lanes b–d are DZQ at 5 μM, pH 7, + 2 mM ascorbic acid (lane b), 10 μM, pH 4 (lane c), and 0.1 μM, pH 4, + ascorbic acid (lane d). Lane e is BZQ at 10 μM, pH 4, and lane f is BZQ at 2 μM, pH 4, + ascorbic acid. Lane g is compound D2 at 100 μM, pH 4, + ascorbic acid, and lane h is compound D1 at 100 μM, pH 4, + ascorbic acid. All reactions were carried out at 20 °C for 1 h. The base sequence number in pBR322 is indicated (for sequence see Figure 5), and arrows indicate the positions of 5′-TGC-3′ within the sequence.

The patterns of alkylation produced were compared qualitatively under conditions of comparable alkylation and at doses that gave at most 1 alkylation/DNA molecule. DZQ and BZQ alkylate all guanines within the DNA sequence under nonreducing conditions (Figure 4, lanes c and e, and corresponding densitometric traces in Figure 5, traces 1 and 2). Not all guanines are alkylated to the same extent within the sequence, and some small but consistent differences are observed between the two compounds (e.g., G_{155}). In general, however, the sequence selectivity is similar to that observed previously for chemotherapeutic agents such as the nitrogen mustards (e.g., melphalan, Figure 5, trace 4; Mattes et al., 1986b).

Following reduction, the pattern of alkylation by BZQ, although quantitatively altered, was qualitatively the same (Figure 4, lane f). The pattern was similar to that obtained for compounds D1–D7 under low-pH/reducing conditions (e.g., compounds D1 and D2, lanes h and g, respectively). The exception was DZQ, which upon reduction at pH 4 gave not

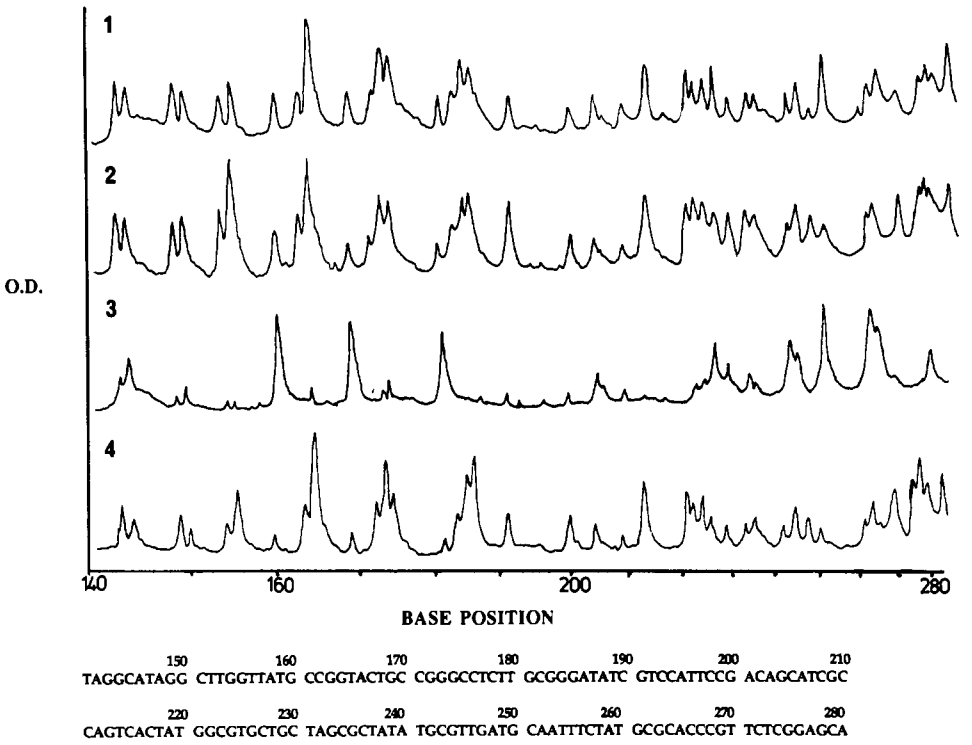

FIGURE 5: Representative densitometric traces of autoradiogram (Figure 4). Trace 1 is DZQ, pH 4 (Figure 4, lane c), trace 2 is BZQ, pH 4 (Figure 4, lane e), trace 3 is DZQ, pH 4, + ascorbic acid (Figure 4, lane d), and trace 4 is the pattern of alkylation observed by the nitrogen mustard melphalan for comparison. In each case the trace is scaled to the highest absorbance value in that lane. The corresponding DNA base sequence of pBR322 is indicated.

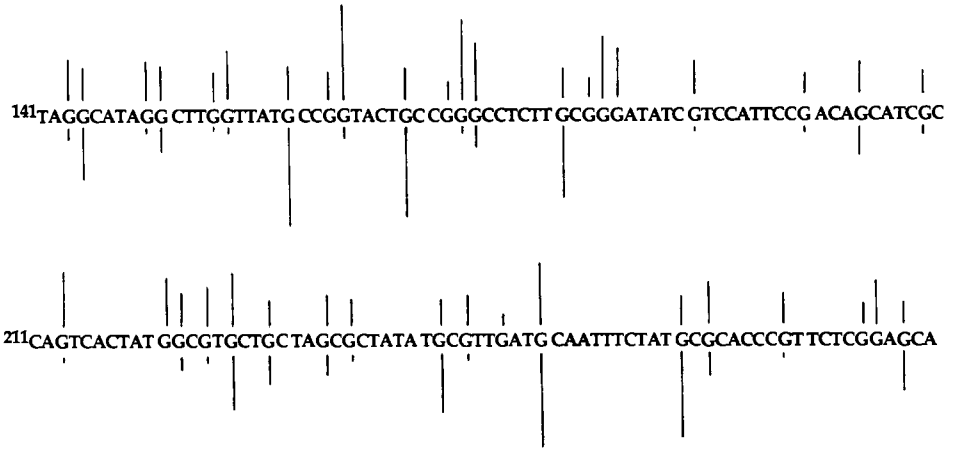

FIGURE 6: Schematic diagram of the pattern of guanine-N7 alkylation by DZQ either in the nonreduced quinone form (above the sequence) or following reduction to the hydroquinone form (below the sequence). Bars indicate the relative intensity of guanine alkylations scaled to the highest alkylation in each case. Data are derived by densitometry from Figure 4, lanes c (10 μM, pH 4) and d (0.1 μM, pH 4, + 2 mM ascorbic acid).

only an approximate 100-fold increase in overall alkylation but also a markedly altered pattern of guanine reactivity (Figure 4, lane d, and Figure 5, trace 3). The same pattern was observed at pH 7 under reducing conditions (lane b) and was due to guanine-N7 alkylation and not due to a drug-induced free-radical mechanism of strand cleavage since the bands produced were only observed when alkylations were converted to strand breaks with hot piperidine and were not observed if the piperidine step was omitted (data not shown). The alkylation observed was restricted to a subset of guanines within the sequence which on close examination were found to be primarily within 5′-GC-3′ sequences. All such sequences were alkylated with 5′-GX-3′ sequences (where X = A, G, or T) alkylated only occasionally, and then very weakly. Not all 5′-GC-3′ sequences are alkylated to the same extent, however, and a clear preference for 5′-TGC-3′ sequences is observed. This can be clearly seen in Figure 4 where the eight occurrences of 5′-TGC-3′ are indicated. Thus DZQ is unique in that the reduced hydroquinone form of the drug has a distinctly different and increased DNA sequence selectivity for guanine-N7 positions than the nonreduced quinone form. This is summarized schematically in Figure 6 for the DNA sequence presented and has been confirmed in several other DNA sequences, and following reduction of DZQ by other chemical and enzymatic methods (data not shown).

Discussion

The reactivities of the diaziridinylbenzoquinones are determined by the ability of the aziridines to undergo ring opening in the presence of nucleophiles and the ease of quinone

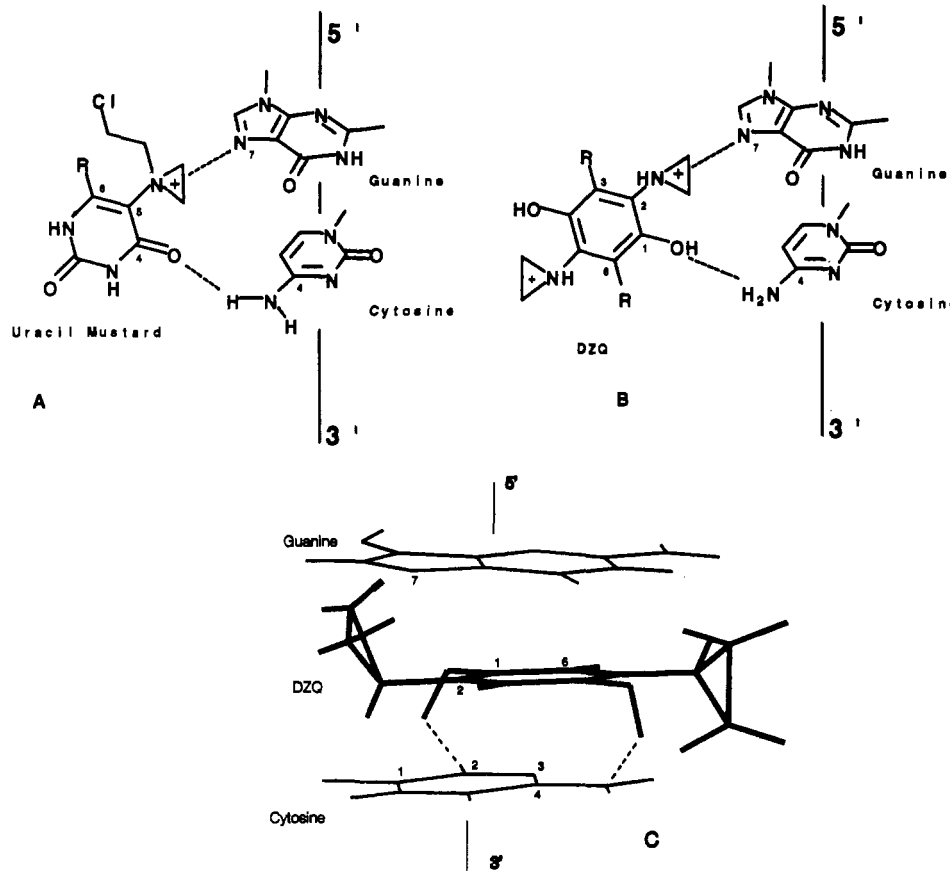

FIGURE 7: (A) Possible configuration of uracil mustard (aziridinium form) at the initiation of the reaction with guanine-N7, showing the proposed interaction of the uracil O4 atom with the NH of a 3'-cytosine [from Kohn et al. (1987)]. Dotted lines indicate hydrogen bonding. (B) A model for the preferential interaction of DZQ (hydroquinone form) at 5'-GC-3' sequences based on that proposed for uracil mustard (panel A). (C) An alternative model for the hydroquinone form of DZQ (bold) located between the base pairs in a 5'-GC-3' sequence. The dotted lines indicate the hydrogen bonding to the cytosine O2 and C4-NH2 groups. The figure is derived directly from computer molecular modeling, but the structures have not been energy minimized.

reduction. Previous studies have shown that BZQ can readily undergo aziridine ring opening and addition to DNA without reduction (Butler et al., 1989). This has essentially been confirmed in the present study (Table I). However, it is interesting to note that, by using the more sensitive agarose gel technique, it has been possible to also demonstrate that reduction at both pH 4 and pH 7 does give rise to a definite increase in the extent of DNA cross-linking. It was previously proposed that reduction of BZQ may be less important in determining the reactivity than acid-catalyzed ring opening. The present results show that, even with BZQ, the increase in electron density when the quinone is reduced to an aromatic semiquinone or hydroquinone still facilitates aziridine ring opening and cross-linking of DNA.

The results in Table I also show that although the overall extent of DNA cross-linking is less with AZQ (D2) and the AZQ analogues (D1, D3–D7), the effect of reduction is more pronounced with this class of compound. The aziridines on these compounds are much more stable than BZQ due to the smaller electron-donating effect of the NHCOO group compared with the $NHCH_2CH_2$ group. The overall trend of DNA cross-linking for the different analogues at pH 4 is similar to that found previously using a different technique and correlates with the ease of reduction of the quinones (Dzielendziak et al., 1990). In addition, the toxicity of compounds D1–D7 to certain cell lines correlated both with the ease of reduction of the compounds and with the efficiency of the compounds to form DNA interstrand cross-links (Dzielendziak et al., 1990). More recently, a trend has also been demonstrated between the rate of reduction of the compounds by the two-electron NAD(P)H:(quinone acceptor) oxidoreductase (DT-diaphorase) and their ability to induce cytotoxicity in the DT-diaphorase-rich HT-29 human colon carcinoma cell line (Gibson et al., 1991).

The simple diaziridinylbenzoquinone (DZQ) has intermediate properties between those of the AZQ analogues and BZQ. This compound is similar to AZQ in that it is easily reduced, $E_7[Q/Q^{\bullet-}] = -54$ mV (K. J. Lusthof et al., submitted for publication), compared to $E[Q/Q^{\bullet-}] = -65$ mV for AZQ (Dzielendziak et al., 1990), and yet readily undergoes acid-catalyzed ring opening in a similar manner to BZQ (Lusthof et al., 1989). The results in Table I clearly demonstrate that at both pH 4 and pH 7, the quinone can cross-link in the absence of reduction. However, the extent of cross-linking, particularly at pH 7, is significantly increased on reduction. The efficiency of cross-linking of a diaziridinylbenzoquinone should also depend on the relative reactivities of the protonated aziridines with DNA or with other nucleophiles including OH^- and buffers (Butler et al., 1989). It is significant that the efficiency of cross-linking for reduced DZQ at both pH 4 and pH 7 is 100% relative to the other compounds. This could be due to the high affinity of the reduced form for certain sites in the DNA (see below), which could lead either to an increased efficiency of cross-linking at these sites compared to the other compounds or alternatively to the formation of cross-links through a different mechanism or with a different sequence specificity. The overall extent of guanine-N7 alkylation follows the same trend as the DNA cross-linking and suggests that it is this base modification which is responsible for the cross-link in a similar manner to other chemothera-

peutic alkylating agents, such as the nitrogen mustards (Mattes et al., 1986b).

Although much more striking, the sequence selectivity of reduced DZQ for 5′-TGC-3′ sequences shows some similarity to the preferential alkylation for 5′-PyGC-3′ sequences (Py = pyrimidine) observed previously for uracil mustard (Mattes et al., 1986b; Kohn et al., 1987). A model for DZQ was considered based on one proposed by Kohn et al. (1987) for the sequence-selective interaction of uracil mustard with a 5′-GC-3′ sequence (Figure 7A). DZQ is similar to uracil mustard in that one alkylating aziridine unit and a hydrogen-bondable oxygen substituent are arranged in adjacent positions on an aromatic ring. DZQ would appear to better fit this model (Figure 7B), as the C1-hydroxyl substituent should be capable of both donating and accepting a hydrogen bond. The C4-quinone carbonyl oxygen of uracil mustard is only capable of accepting a hydrogen bond. However, this model does not account for the fact that the quinone form of DZQ does not show the sequence selectivity that would be expected on the basis of the much closer analogy to uracil mustard.

On the basis of this problem, an alternative model was explored. Figure 7C shows DZQ in the hydroquinone form, intercalated between the guanine and cytosine residues of one DNA strand. According to molecular modeling studies, even with the molecule positioned equidistant between the two bases, protons of the two hydroxyls of the hydroquinone are within favorable bonding distance of the cytosine O2 and C4-NH_2 groups (approximately 0.9 and 1.28 Å, respectively). Moving the drug closer to the cytosine improves these hydrogen bond distances. Such interactions could not occur with the quinone form of the drug. Another important feature of this model is that the reactive carbon of the aziridine is positioned within covalent bond forming distance of the N7 position of the guanine residue above the plane of the drug, suggesting that the hydrogen bonds hold the drug in the appropriate orientation for covalent bond formation. The model also supports the observed GC sequence selectivity, as these hydrogen-bonding interactions would not be possible with purines (e.g., GG or GA sequences). In the case of thymine (e.g., GT), hydrogen bonding as described above would still be possible to the O2 and O4 groups. However, it is clear from the model that some steric interaction would result from the C5-CH_3 group of thymine. A less likely explanation for GC rather than GT selectivity is that the drug hydrogen bonds to cytosine via hydrogen bond donation to O2, but with C4-NH_2 acting as a hydrogen donor to the oxygen of the other hydroquinone hydroxyl. If this were the case, overall hydrogen bonding to thymine would be reduced, as the hydrogen-donating properties of C4-NH_2 would not be available. However, due to the symmetry of the drug, there is no apparent reason why this mode of hydrogen bonding should be more favorable than that involving hydrogen donation by both hydroquinone hydroxyls.

This latter model also indicates that, following covalent bonding of the drug to the guanine-N7 position, the hydrogen bonds are not significantly disturbed (now approximately 1.1 and 1.0 Å) and that the N–C–C link joining the drug to guanine is in a low-energy conformation with negligible steric interaction with surrounding groups. Also predicted is that the sequence specificity would not occur as readily with the other diaziridinylbenzoquinones as the more bulky side groups in the 3- and 6-positions may lead to steric interactions with the guanine and cytosine above and below the molecule. Previous studies with simple analogues of DZQ have also shown that bulky side groups in both the 3- and 6-positions have lower cross-linking efficiencies (Lusthof et al., 1989). However, it is not clear from our present model whether the sequence specificity could still be maintained when one of the side groups remains as a hydrogen while the other one is modified. Compounds of this type would have the added advantage in that, depending on the structure of the side group, the redox capability of the quinone could be modified. Nonsymmetric compounds are now being synthesized.

Acknowledgments

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