 Nucleic Acids Research, 1999, Vol. 27, No. 13 **2601–2609**

# Spermine inhibition of the 2,5-diaziridinyl-1,4-benzoquinone (DZQ) crosslinking reaction with DNA duplexes containing poly(purine)·poly(pyrimidine) tracts

**Tatyana Shamma and Ian S. Haworth***

Department of Pharmaceutical Sciences, University of Southern California, Los Angeles, CA 90033, USA

Received April 8, 1999; Accepted May 10, 1999

## ABSTRACT

**Upon reduction, 2,5-diaziridinyl-1,4-benzoquinone (DZQ) can form an interstrand guanine to guanine crosslink with DNA duplexes containing a d(GC)·d(GC) dinucleotide step. The reaction is enhanced by a thymine positioned 5' to each guanine [i.e. in a d(TGCA)·d(TGCA) duplex fragment]. Here we show that spermine can inhibit DZQ crosslink formation in duplexes of sequence d[C($N_6$)TGCA($M_6$)C]·d[G($M'_6$)TGCA($N'_6$)G]. For $N_6$ = $M_6$ = GGGGGG, $N_6$ = $M_6$ = a 'random' sequence and $N_6$ = GGGGGG and $M_6$ = a 'random' sequence, spermine concentrations of 20, 1 and 3 μM, respectively, were required for 50% inhibition of the DZQ crosslink. This suggests that spermine is more strongly bound to the polyguanosine tract than the random sequence, making it less available for crosslink inhibition. When the polyguanosine tract is interrupted by *N*7-deazaguanine (D) located three bases, d(CGGGDGGTGCAGGDGGGC), and four bases, d(CGGDGGGTGCAGGGDGGC), from the d(TGCA)·d(TGCA) site, 30 and 3 μM spermine, respectively, were required for 50% crosslink inhibition. We suggest that this difference is due to the relative proximity of the three-guanosine tract to the d(TGCA)·d(TGCA) site. We were able to confirm these conclusions with further experiments using duplexes containing three-guanosine and two-guanosine tracts and from computer simulations of the spermine–DNA complexes.**

## INTRODUCTION

Natural polyamines such as spermine (Fig. 1a), spermidine [$H_2N(CH_2)_3NH(CH_2)_4NH_2$] and putrescine [$H_2N(CH_2)_4NH_2$] are found in both prokaryotic and eukaryotic cells. They play an important role in cell growth and differentiation (1–3) and their concentration tends to elevate in rapidly proliferating cells, such as those found in tumors (4). Conversely, depletion of natural polyamine levels inhibits cell proliferation (5,6). At physiological pH the natural polyamines are polycationic. Hence, they have a strong electrostatic association with DNA and may be important in facilitating the formation of nucleosome structures (7). Polyamines stabilize duplex DNA against denaturation (8) and have also been shown to modulate the interaction of DNA with restriction endonucleases (9) and with a number of other DNA-binding proteins (10–13). Polyamines also stabilize other DNA conformations, such as A-DNA (14) and triplex DNA (15–17), and promote the transition of DNA from a right-handed to a left-handed duplex, the so-called B to Z transition (18–20). One key possible biological role of polyamines may be their ability to condense DNA (19,21) and chromatin (22,23).

Despite the above observations, the molecular details of the polyamine interaction with DNA are not well understood. One particularly interesting question is whether the interaction is better represented as a purely ionic association or whether it also has characteristics (such as base sequence selectivity) more similar to those of a ligand–DNA complex. One approach that has been used to explain the polyamine–DNA association is counterion condensation theory (24; for a recent review see 25), in which the polyamines are assumed to behave as point charges. This description has successfully reproduced many aspects of the polyamine–DNA interaction, although it lacks any structural and/or sequence-specific component. However, there is evidence that the exact structure of the polyamine is important in the efficiency with which, for example, DNA is aggregated (19).

Theoretical models have favored spermine binding to GC-rich major grooves (26–28) and AT-rich minor grooves (29). This selectivity is supported by spermine inhibition of GC major groove-specific DNA alkylating agents (30) and from experiments on photoreactive polyamine derivatives, which indicated an AT-rich, minor groove binding mode (31). Consistent with this, evidence for sequence-selective spermine–DNA base interactions has been reported (32), but, in contrast, solution studies (33,34) suggest non-specific modes of binding of polyamines to the phosphate backbone of the DNA. Association of spermine with DNA has also been observed in several X-ray structures, but different locations for spermine have been reported. Spermine spans a GC-rich major groove in a fragment of the *gal* operon (35) and in the Dickerson-Drew dodecamer $[d(CGCGAATTCGCG)]_2$ (36), interacting with the phosphates of the opposite strands in each case. Spermine has also been located in the major groove of several A-DNA structures (37–39). Of note in these structures, in addition to the location, is the spermine conformation, which may be extended (essentially all *trans*) (37), or 'U-shaped' (a spermine with sharp bends at both ends of its structure) (38). Such U-shaped conformations

*To whom correspondence should be addressed. Tel: +1 323 442 3310; Fax: +1 323 442 1390; Email: ihaworth@hsc.usc.edu

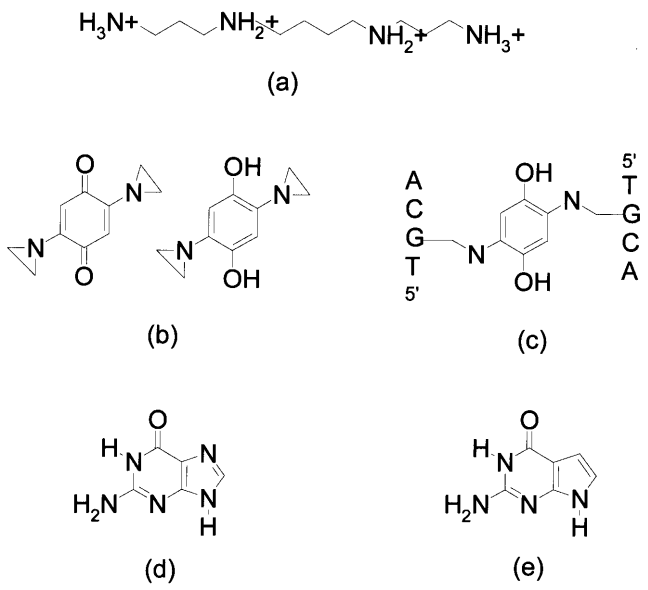


**Figure 1.** Structures of (**a**) the spermine tetracation, (**b**) the oxidized and reduced forms of 2,5-diazridinylquinone (DZQ), (**c**) the DZQ 1,2 guanine–guanine crosslink at a d(TGCA)·d(TGCA) sequence, (**d**) guanine and (**e**) *N*7-deazaguanine.

have also been observed in spermine complexes with Z-DNA (40) and with a DNA duplex containing an intercalated anthracycline (41).

Here we have used a new approach to determine if spermine has a sequence-selective component to its binding with DNA. This approach is based on the ability of spermine to inhibit (30) the guanine N7 to guanine N7 1,2-crosslink formed by the reduced form of 2,5-diazridinylquinone (DZQ) (Fig. 1b and c) in the DNA major groove (42–44). We show that the inhibition of the crosslink in duplexes containing a central d(TGCA)·d(TGCA) crosslink site (the preferred DZQ crosslinking site) is dependent on the sequences flanking the crosslink site and interpret these data in terms of differential spermine interactions with the flanking sequences. Using this approach, we suggest that a run of at least three guanine bases is required for efficient spermine binding to a polyguanosine tract and that the spermine interaction with such tracts probably occurs in the major groove with the guanine N7 atom.

## MATERIALS AND METHODS

### Experimental method

*Chemicals and reagents.* Spermine tetrahydrochloride and $T_4$ polynucleotide kinase were purchased from Sigma Chemical Co. [$\gamma$-$^{32}$P]ATP was purchased from ICN. *N*7-deazaguanine was purchased from Glen Research. DZQ was obtained from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute. All oligonucleotides were synthesized on an Applied Biosystems DNA automated synthesizer (model 391), deprotected with saturated ammonium hydroxide and purified on a COP cartridge in the Microchemical Core Facility of the USC Norris Cancer Center. All other reagents were at least of analytical grade and used without further purification.

*$^{32}$P 5′-end-labeling of DNA.* The 5'-end of one strand (10 μM) of each synthetic oligonucleotide duplex was $^{32}$P-radiolabeled by incubation for 1 h at 37°C with 10 U of $T_4$ polynucleotide kinase and [$\gamma$-$^{32}$P]ATP (5 μl, 4500 Ci/mmol) in labeling buffer solution (50 μl). The reaction was stopped by addition of sodium acetate (0.6 M, pH 5.2) and was immediately precipitated in three times the volume of cold 95% ethanol at –20°C overnight. The unincorporated [$\gamma$-$^{32}$P]ATP and labeling buffer were decanted. The DNA pellet was dried in a SpeedVac for 15 min and stored at –20°C for further use.

*Annealing of DNA.* An equal amount of unlabeled complementary strand was added to the labeled oligonucleotide in double deionized water, heated to 65–70°C and then gradually cooled to room temperature overnight to form the annealed duplex.

*DNA crosslinking competition reactions.* Duplexes (1 μM) were incubated with 0, 0.5, 5, 50, 500 or 5000 μM spermine at 37°C for 1 h and subsequently with DZQ (200 μM) for 2 h. The incubations were performed in 50 μl of potassium phosphate solution (10 mM, pH 5.8) containing NADH (100 μM), 12.8 mg/ml stock of DT-diaphorase and 1 mM EDTA. The reaction was then immediately terminated by the addition of 5.5 μl of sodium acetate (3 M). The DNA was precipitated in three times the volume of cold 95% ethanol and 5 μl of tRNA at −20°C overnight. The DNA was then washed, dried in a SpeedVac and dissolved in 2 μl of distilled deionized water and 8 μl of tracking dye (80% formamide, 1 mM EDTA, 0.025% bromophenol blue and xylene cyanol).

*Detection and quantification of guanine to guanine interstrand 1,2-crosslinks.* The samples were loaded onto a 20% denaturing polyacrylamide gel (29:1 monoacrylamide:bisacrylamide, 8 M urea, 0.4 mm thickness, 38 × 31 cm, 2500 V, 50 W) and allowed to run until the xylene cyanol marker had migrated 10 cm. The gel was exposed to X-ray film and the percentage of guanine to guanine DNA interstrand 1,2-crosslinks in the duplex oligonucleotide was determined using Kodak Digital Science 1D software (Kodak Scientific Imaging Systems). For a given spermine concentration, the intensity of the crosslink band was calculated as a fraction of the total DNA in the lane. These numbers for different spermine concentrations were then related to each other using a scale based on 100% crosslinking at zero spermine concentration. Experiments were performed at least three times for each DNA duplex.

### Computational method

*Solvated molecular dynamics simulation of spermine.* A fully solvated molecular dynamics simulation of the spermine tetracation was performed using AMBER 4.0 (45) as follows. *Trans*-spermine (all the C-C and C-N bonds included as *trans* conformers) was solvated in a periodic box of TIP3P water molecules. The parameters and charges for spermine have been published elsewhere (30). The box had dimensions 40 (the axis corresponding to the spermine long axis) × 40 × 40 Å. Following brief minimizations (200 cycles) of the water and the spermine, respectively, a simulation of 70 ps was performed in the nPT ensemble, using a time step of 0.002 ps and a non-bonded

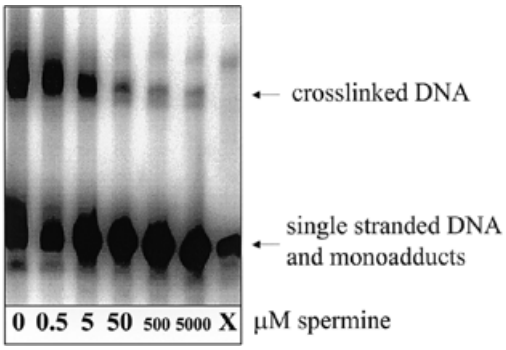


**Figure 2.** A typical autoradiogram of a 20% denaturing PAGE gel showing spermine inhibition of a DZQ crosslink for the guanine-rich sequences shown in Table 1. The gel shows electrophoresis of the products of incubation of 200 μM DZQ with the duplex d(CGGDGGGTGCAGGGDGGC)·d(GCCCCCCTGCACCCCCCG), in which the guanine-rich strand was radiolabeled. Prior to this, the duplex had been pre-incubated with spermine at the concentrations indicated for each lane. Lane X is a control lane with no DZQ and no spermine.

cut-off of 8 Å, at a temperature of 298 K. The temperature was increased linearly from 0 to 298 K in the first 10 ps of the simulation. The final structure from this simulation was resolvated in a similar periodic box and an identical 70 ps simulation was performed from this starting point.

*Simulations of spermine–DNA complexes.* Molecular dynamics simulations were performed using the AMBER 4.0 force field (45). Standard AMBER 4.0 parameters and charges were applied to the DNA. B-DNA helices were constructed using the QUANTA 4.0 package (46). The starting position of the spermine was unbiased toward any binding site and was ~10 Å from the DNA. In the simulation, the spermine molecule diffused initially towards the DNA and then moved freely over the surface. A light constraint of 1 kcal/mol/Å was used to maintain a 5 Å distance between the primary amine nitrogens of the spermine (for the basis of this see Results). Simulations were performed at 298K for 1 ns on all systems, using a time step of 0.002 ps. A distance-dependent dielectric ($\varepsilon = 4r$) was used to reproduce the effect of solvent on the electrostatic interactions and the DNA motion was frozen throughout the simulations. Coordinates were saved every 0.4 ps to allow for subsequent analysis of the spermine motion relative to the DNA duplex.

## RESULTS

### The DZQ guanine–guanine 1,2-crosslink is inhibited by spermine

DNA duplexes of sequence d($CN_6TGCAM_6C$)·d($GM'_6TGCAN'_6G$), where $N_6$ and $M_6$ are as shown in Table 1 (and $M'_6$ and $N'_6$ are complementary to $M_6$ and $N_6$, respectively) were equilibrated with serially diluted concentrations of spermine, followed by incubation with DZQ. The resultant interstrand crosslinked DNA duplexes were separated on a polyacrylamide gel from the unreacted single-stranded DNA and DNA monoadducts (Fig. 2) and quantified by densitometry. For each spermine concentration, the intensity of the crosslink band was expressed as a percentage of the total DNA in the lane on the gel. The efficiency of the crosslinking reaction in the absence of spermine is similar for all the duplexes shown in Table 1, with ~30% of the total DNA being crosslinked in each case (Fig. 3D). For each duplex, the level of crosslinking at zero spermine concentration was considered to represent 100% crosslinking and the efficiency of the crosslinking reaction at increasing concentrations of spermine was then expressed as a percentage of this maximal level of crosslinking. This allowed a comparison of the efficiency of spermine inhibition of crosslinking with each duplex.

**Table 1.** Spermine inhibition of DZQ crosslinking in different DNA duplexes[a]

| Top Strand of Duplex (5' - 3') [b] | Shorthand Notation | % Crosslinking at 5μM spermine | [Spermine] (μM) for 50% inhibition |
|---|---|---|---|
| C**GGGGGG***TGCA***GGGGGG**C | GG | 85 | 20 |
| C**GTAACA***TGCA***TCGAAT**C | RR | 52 | 1 |
| C**GGGGGG***TGCA***TCGAAT**C | GR | 65 | 3 |
| C**GGGDGG***TGCA***GGDGGG**C | DD4 | 88 | 30 |
| C**GGDGGG***TGCA***GGGDGG**C | DD3 | 65 | 3 |
| C**GGGTAC***TGCA***CATGGG**C | 123G | 92 | nd [c] |
| CC**GGGTA***TGCA***ATGGGC**C | 234G | 84 | nd |
| C**ACGGGT***TGCA***TGGGCA**C | 345G | 81 | nd |
| C**TACGGG***TGCA***GGGCAT**C | 456G | 72 | nd |
| C**GGDTAC***TGCA***CATDGG**C | 12G | 74 | nd |
| CC**DGGTA***TGCA***ATGGDC**C | 34G | 73 | nd |
| C**TACDGG***TGCA***GGDCAT**C | 56G | 73 | nd |
| C**AAAAAA***TGCA***AAAAAA**C | AA | 100 | see text [d] |

[a]Based on the average of at least three experiments for each duplex.
[b]D = *N*7-deazaguanine, underlined bases are three-guanosine and two-guanosine tracts and double-underlined bases are guanine to *N*7-deazaguanine substitutions (in, for example, duplex 12G, compared to duplex 123G).
[c]Not determined
[d]The spermine inhibition curve for duplex AA does not follow that of the other duplexes (see text and Figs 3C and 4A).

### The spermine concentration required to inhibit the DZQ crosslink is dependent on the duplex sequence neighboring the d(TGCA)·d(TGCA) crosslink site

A comparison of the inhibition of the DZQ crosslink as a function of spermine concentration is shown for duplexes GG, RR and GR in Figure 3A and in Table 1. In interpreting these data, we consider that the variation in inhibitory effect of the spermine provides us with indirect information on the affinity of spermine for the flanking sequences. Hence, our assumption is that higher affinity binding to the $N_6$ and $M_6$ tracts would result in less spermine inhibition of the crosslink in the central

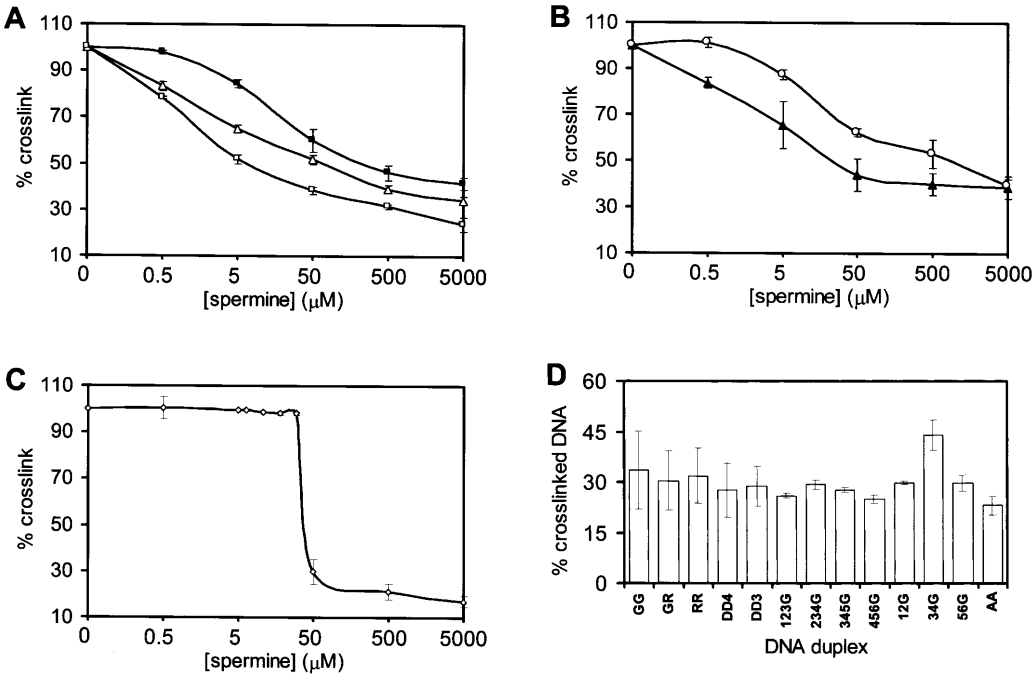


**Figure 3.** Spermine inhibition of DZQ crosslinking of (**A**) duplexes GG (closed square), GR (open triangle) and RR (open square), (**B**) duplexes DD3 (closed triangle) and DD4 (circle) and (**C**) duplex AA. The full sequences of each duplex are given in Table 1. (**D**) The percentages of DNA crosslinked by DZQ in the absence of spermine are shown for all the duplexes listed in Table 1. In each figure the error bars are standard deviations from the mean.

d(TGCA)·d(TGCA) site. Inhibition of crosslinking in duplex GG as a function of spermine concentration followed a sigmoidal shape, with 50% inhibition requiring ~20 μM spermine (Fig. 3A and Table 1). In contrast, the random sequence (RR) required only 1 μM spermine for 50% inhibition (Fig. 3A and Table 1). This result is similar to that found previously for a random sequence 20mer, d(CTTCCAAGATGCATCAGATG)·d(CATCTGATGCATCTTGGAAG), containing a similar d(TGCA)·d(TGCA) crosslink site (30). For GR, a part polyguanosine, part random sequence duplex, an intermediate value of 3 μM spermine was required to achieve 50% inhibition of crosslinking (Fig. 3A and Table 1). Hence, 50% inhibition of the DZQ crosslink for GG required a 6-fold greater spermine concentration (20 μM) than that for GR (3 μM), which in turn required a 3-fold increase over that for RR (1 μM). These results suggest a higher affinity interaction of spermine with the polyguanosine tract, compared to the random sequence DNA.

### Interruption of the polyguanosine tract by *N*7-deazaguanine influences the spermine inhibitory effect on the DZQ crosslinking reaction

To determine if the spermine interaction with the polyguanosine tract might occur through the major groove, and specifically with the guanine N7 atom, experiments were performed on duplexes DD3 and DD4 (Table 1). These duplexes have the polyguanosine tract interrupted by an *N*7-deazaguanine (D) base (Fig. 1e) located at positions 3 and 4 of each polyguanosine tract, respectively [where the tracts are numbered such that guanine 1 is distal to the d(TGCA)·d(TGCA) site]. For both DD3 and DD4, inhibition of crosslinking as a function of spermine concentration is approximately sigmoidal, as for the GG duplex (Fig. 3B). For DD3, 50% inhibition of the DZQ crosslink required ~3 μM spermine, consistent with what might be expected for reduced spermine binding affinity for the polyguanosine tract caused by the presence of an *N*7-deazaguanine. However, for DD4, an increase in spermine concentration (30 μM) is required for 50% crosslink inhibition, compared to that for GG (20 μM).

### The proximity of a three-guanosine tract to the d(TGCA)·d(TGCA) crosslink site influences the spermine inhibitory effect on the DZQ crosslinking reaction

The main difference between the DD3 and DD4 duplexes (Table 1) is the position of the two-guanosine and three-guanosine tracts relative to the crosslinking site. To test the conclusion that the proximity of the three-guanosine tract to the d(TGCA)·d(TGCA) crosslink site is important in determining the inhibitory effect of the spermine, we designed four different DNA duplexes that have a three-guanosine tract and where, in successive duplexes, the tract is moved one base closer to the d(TGCA)·d(TGCA) site (duplexes 123G, 234G, 345G and 456G; Table 1). In designing these duplexes we maintained the same flanking bases to the 5'- and 3'-side of the three-guanosine tract. We note that this creates a potential d(GC)·d(GC) crosslink site additional to that of the favorable central d(TGCA)·d(TGCA) crosslink site, but in none of these experiments was any significant level of DNA crosslinking apparent at the

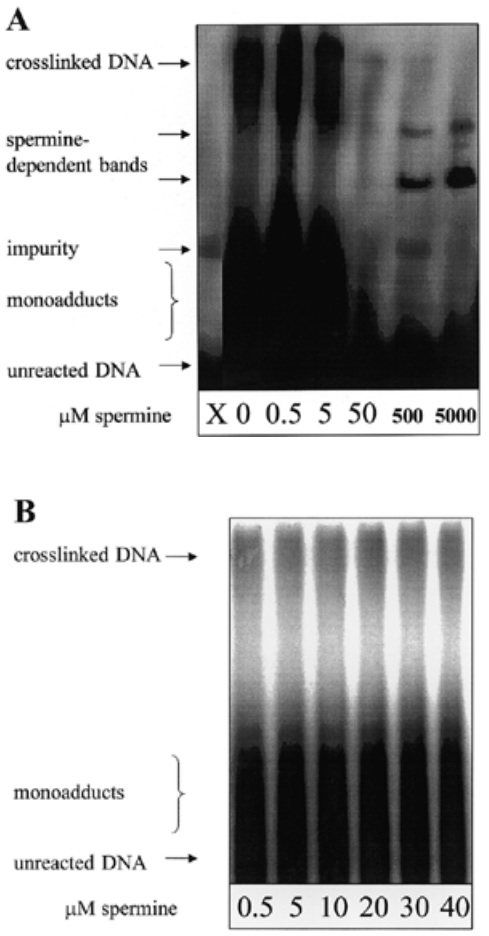


**Figure 4.** Autoradiodiagrams of 20% denaturing PAGE gels showing the effect of spermine on the DZQ crosslinking reaction with d(CAAAAAATGCA-AAAAAAC)·d(GTTTTTTTGCATTTTTTG). (**A**) The spermine concentration ranges from 0 to 5000 μM, as indicated, and lane X is a control lane with no DZQ and no spermine. (**B**) Results are shown for a narrower concentration range of spermine.

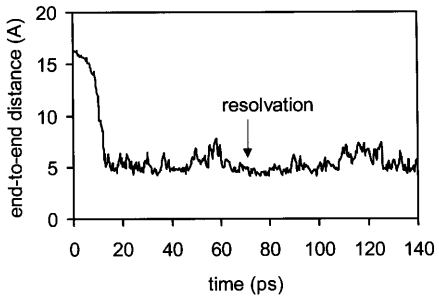


**Figure 5.** The distance between the primary amines of a spermine tetracation over 140 ps of fully solvated molecular dynamics simulation, beginning from an all *trans* spermine conformer. The resolvation label refers to interruption of the calculation to resolvate the spermine conformer generated after 70 ps of simulation (see text for details).

second site (data not shown). We note further that the efficiency of crosslinking for each of these duplexes in the absence of spermine is comparable with that for duplex GG (Fig. 4D). Each of the duplexes was equilibrated with 5 μM spermine, followed by the addition of DZQ. The crosslink band at 5 μM spermine decreases in intensity as the three-guanosine tract is positioned closer to the d(TGCA)·d(TGCA) crosslink site (Table 1). This suggests that the three-guanosine tract acts as a relatively high affinity binding site for spermine, compared to the rest of the duplex sequence, and can influence the overall distribution of spermine on the duplex.

### The position of a two-guanosine tract relative to the d(TGCA)·d(TGCA) crosslink site has no influence on the spermine inhibitory effect on the DZQ crosslinking reaction

To examine the influence of the position of a two-guanosine tract on the DZQ crosslinking reaction, duplexes 12G, 34G and 56G (Table 1) were used. These sequences are identical to those of duplexes 123G, 234G and 456G, respectively, except that a single guanine to *N*7-deazaguanine replacement has been made in each three-guanosine tract, creating three sequences with differently positioned two-guanosine tracts (Table 1). Spermine at 5 μM inhibits DZQ crosslinking to a similar extent in duplexes 12G, 34G and 56G, suggesting that, in this series of duplexes, the location of the two-guanosine tract is insignificant in determining the inhibition efficiency.

### Spermine is unable to inhibit DZQ crosslinking of a duplex containing polyadenosine tracts at low concentration, but does so efficiently at high concentration

We also examined the inhibition effect of spermine with a duplex containing polyadenosine tracts (duplex AA, Table 1). With duplex AA, there was no gradual decrease in crosslinking efficiency as a function of spermine concentration. In contrast, the intensity of the crosslink band was essentially unchanged with 0.5 and 5 μM spermine, but underwent a sharp decrease at higher (≥50 μM) spermine concentrations (Fig. 3C). Additional data obtained at 10, 20, 30 and 40 μM spermine (Fig. 3C) showed that there is essentially no inhibition of crosslinking up to 40 μM spermine for duplex AA. Gels showing the results of DZQ crosslinking of duplex AA in the presence of spermine are shown in Figure 4. At 50 μM spermine the appearance of new bands (labeled spermine-dependent bands in Fig. 4A) suggests that a possible DNA conformational change has occurred and that this might be the origin of the crosslink inhibition for this duplex. These bands are even more clearly seen at higher spermine concentrations and the data in Figure 4A are in contrast to those typically obtained for guanine-rich duplexes (Fig. 2). From Figure 4B it is apparent that the crosslinking reaction is essentially unchanged by spermine concentrations up to 40 μM.

### In a solvated molecular dynamics simulation, spermine adopts a 'U-shaped' conformation

To gain more understanding of the spermine–DNA association, we performed a series of simulations using duplexes of the same sequence to those used experimentally. However, prior to performing simulations on the DNA–spermine complexes, we wanted to assess the likely conformation of spermine in solution. To do this, we performed solvated molecular dynamics simulations on the tetracation alone. The key result from this

**Table 2.** Summary of site occupancy in simulations of spermine with DNA duplexes GG and RR

| Duplex | d(TGCA)·d(TGCA)[a] (%) | G tract[b] (%) | Major groove[c] (%) | Minor groove[c] (%) |
|---|---|---|---|---|
| GG | 3.6 | 85.7 | 91.6 | 8.4 |
| RR | 15.0 | | 26.7 | 73.2 |

In the occupancy calculations the spermine location is defined by the midpoint between the N5 and N10 atoms of spermine.
[a]Occupancy is defined as spermine being within 8 Å of either O6 atom of the guanines of the d(TGCA)·d(TGCA) site.
[b]Occupancy is defined as spermine being within 8 Å of any N7 atom of the polyguanosine tract.
[c]Occupancy of the respective grooves was assessed by calculating the distance between spermine and the closest major groove and closest minor groove 'marker' atom [N6(A) or O6(G) and H2(A) or N2(G), respectively]. The lower of these two distances defines the groove occupancy.

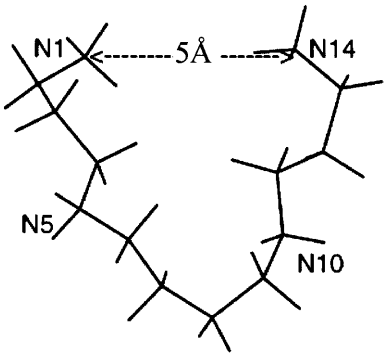


**Figure 6.** A representative 'square planar' (with respect to the amine locations) or 'U-shaped' spermine conformation generated in a solvated molecular dynamics simulation of the spermine tetracation.

simulation is shown in Figure 5. This shows that, from a starting all *trans* conformation that has a distance of ~17 Å between the terminal primary amines, the spermine tetracation folds into a 'U-shaped' conformation in which the four amines adopt a 'square planar' arrangement and the terminal primary amines are ~5 Å apart (Fig. 6). This motion is driven by the formation of hydrophobic interactions between the alkyl chains of the spermine and occurs despite the charges on the terminal amines. Following re-solvation of the 'square planar' conformation after 70 ps of simulation, a second simulation was performed to determine if the conformation would be retained. As shown in Figure 5, the end-to-end distance remains constant over the second simulation (70–140 ps). We thus believe that this conformation reflects the favored spermine conformer in solution and we chose to use the 'square planar' conformer in subsequent simulations of the spermine–DNA complexes. This conformation is similar to that observed in several crystal structures (see Introduction).

### Spermine occupies the major groove of a polyguanosine tract in molecular dynamics simulations

One nanosecond molecular dynamics simulations of spermine with GG and RR (sequences in Table 1) were performed. During these simulations, the spermine migrated freely over the surface of the DNA. The spermine occupancy time in the d(TGCA)·d(TGCA) site was much greater with RR than with GG (Table 2), in agreement with the experimental inhibition data (Fig. 3A and Table 1). With GG, spermine was located in the major groove of the polyguanosine tract for most of the simulation, but adopted no fixed binding site or binding orientation. In contrast, with RR, spermine occupied sites other than in the major groove, largely associated with the backbone, and bound to both the minor and the major groove sides of the backbone. The minor groove occupancy time for RR (Table 2) largely reflects this kind of binding mode. Much of the major groove occupancy time for RR, in contrast, reflects an association with the d(TGCA)·d(TGCA) site (Table 2), consistent with the experimental crosslink inhibition data (Table 1). Hence, although these simulations represent an oversimplification of the real system and, in the absence of experimental data, could not be used to draw independent conclusions, they do provide some insight into how the spermine might associate with the DNA.

### The spermine occupancy of three-guanosine tracts in computer simulations is consistent with experimental results

Simulations with 123G, 234G, 345G, 456G, DD3 and DD4 show that spermine has a tendency to bind more favorably towards the center of the duplex (presumably because of the more favorable electrostatic environment). However, the association time of spermine with the 'isolated' three-guanosine tracts of these duplexes, compared to that with the three-guanosine tract in the same location [relative to the d(TGCA)·d(TGCA) site] in duplex GG is different (Table 3). When the three-guanosine tract is distal to the central crosslink site (in duplex 123G) the spermine is displaced from the central region of the duplex for an ~2-fold longer time, compared to the GG simulation (contrast the 7.8% occupancy of the three-guanosine tract in 123G with the 4.0% occupancy of the outermost three-guanosine tract in GG; Table 3). It is of note that a qualitatively similar result to that with duplex 123G is obtained with duplex DD4, relative to duplex GG. This shows that incorporation of the *N*7-deazaguanine base (and, hence, elimination of the N7 binding site for that base) is sufficient to reduce the affinity of spermine for the modified guanine base and to 'isolate' the three-guanosine tract in DD4. The greater displacement of the spermine from the d(TGCA)·d(TGCA) site accounts for the reduced inhibition of crosslinking in DD4 and 123G, compared to GG (Table 1). A

**Table 3.** Summary of spermine occupancy time of three-guanosine tracts in simulations of spermine with different DNA duplexes

| Duplex | Occupancy of GGG tract (%) | Occupancy of the same GGG in a six-guanosine tract[a] (%) |
|---|---|---|
| 123G | 7.8 | 4.0 |
| 234G | 52.9 | 19.8 |
| 345G | 54.2 | 36.0 |
| 456G | 77.1 | 25.4 |
| DD4 | 31.5 | 4.0 |
| DD3 | 82.7 | 25.4 |

[a]Occupancy is defined by the midpoint between the N5 and N10 atoms of spermine being within 8 Å of the N7 atom of the central guanine base of the GGG tract.

similar comparison can be made between duplexes 456G and DD3, in which the three-guanosine tract is proximal to the d(TGCA)·d(TGCA) crosslink site. The relatively similar tract occupancies (Table 3) by spermine in these two duplexes again suggests that the *N*7-deazaguanine base is missing the key site for spermine binding. Furthermore, the 3-fold increase in spermine occupancy in the three-guanosine site proximal to d(TGCA)·d(TGCA), compared to the occupancy of the same site in the simulation with GG (Table 3), is consistent with increased crosslink inhibition of spermine with 456G and DD3, compared to that with GG (Table 1).

## DISCUSSION

It is apparent from the above results that spermine inhibition of the DZQ crosslinking reaction is dependent on the sequences flanking the d(TGCA)·d(TGCA) crosslink site. Our interpretation of these data is based on a differential association of spermine with the different flanking regions and, hence, the results provide an indirect way of assessing the selectivity of the spermine–DNA interaction. Based on extrapolation from the measured equilibrium binding constants for the spermine–DNA association (47), it is likely that, at 5 μM spermine and 1 μM DNA duplex, under the low counterion concentration conditions used (10 mM $K^+$), almost all the spermine is associated with the DNA [we note that this conclusion is based on original data derived from large DNA molecules (47) and electrolyte association constants are predicted to be significantly reduced, in comparison, for short oligomers (48)]. However, assuming that, at 5 μM spermine, most of the ligand is bound to the DNA, we believe the key to interpreting the data lies in the strength of the spermine interaction with the flanking sequence, relative to the crosslinking site and, if this association is strong, whether it 'concentrates' the spermine proximal or distal to the crosslink site. This will be further discussed below.

We note that there are other possible explanations for the inhibition data. One such explanation could be that the spermine can induce subtle, sequence-dependent DNA conformational changes, which, in turn, could lead to DNA duplex conformations that are more or less easily crosslinked. Indeed, the results with duplex AA are suggestive of such a conformational change at ~50 μM spermine. However, excluding this latter duplex, we believe that it is unlikely that conformational effects could, for example, reasonably explain the differences between duplexes GG, DD3 and DD4, because these duplexes have such similar sequences. Furthermore, as will be shown below, the consistency across a large number of DNA sequences provides us with confidence that the interpretation of the data in terms of spermine affinity for the flanking sequences is correct.

Hence, we believe the results across all the guanine-rich duplexes (Table 1) are consistent with a spermine–DNA interaction with a polyguanosine tract that occurs in the major groove and involves the N7 atom of guanine. This conclusion is based on the effect on spermine inhibition of DZQ crosslinking of interruption of a polyguanosine tract with a *N*7-deazaguanine base. We interpret the results from duplexes DD3 and DD4 as being indirect evidence that a binding site of at least three contiguous guanine bases are required for efficient spermine binding to a polyguanosine tract. In computer simulations, the three-guanosine tract in DD4 localizes the spermine distal to the d(TGCA)·d(TGCA) crosslink site, whereas the equivalent tract in DD3 localizes the spermine proximal to the crosslink site and subsequently allows for more residual binding of spermine in, or close to, the crosslink site. This results in a much lower concentration of spermine required for crosslink inhibition in duplex DD3, compared to DD4.

This conclusion is supported by the increasing amount of spermine required to inhibit DZQ crosslink formation as the three-guanosine tract is located farther from the crosslink site in the series of duplexes 456G, 345G, 234G and 123G, respectively. Further, the position of the two-guanosine tract in duplexes 12G, 34G and 56G has no influence on the spermine inhibitory effect on the DZQ crosslink, which suggests that the two-guanosine tract does not interact strongly with spermine. Since the two-guanosine tracts in duplexes 12G, 34G and 56G were 'created' from the three-guanosine tracts in duplexes 123G, 234G and 456G (Table 1) by substituting a guanine base for a *N*7-deazaguanine base, the different behavior in the two series of duplexes suggests an association between spermine and guanine N7. We note that such an amine–N7 interaction has recently been reported in a DNA–ligand crystal structure (49).

Spermine in an all *trans* conformation could potentially span as many as 5 bp (based on a distance of ~17 Å between the

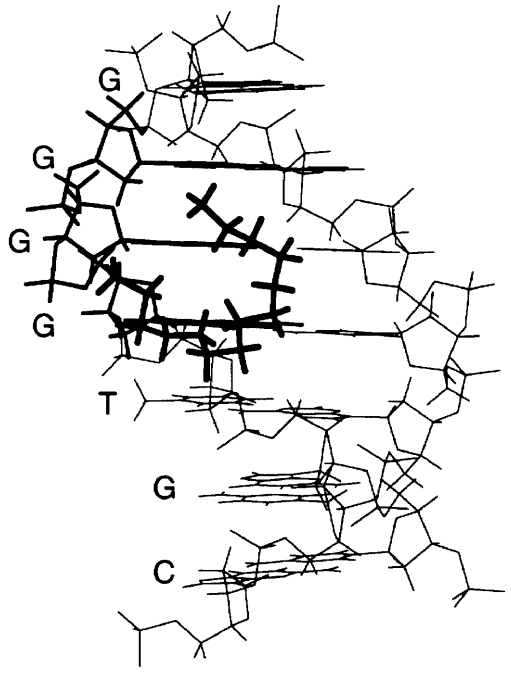


**Figure 7.** A representative structure of those observed in molecular dynamics simulations of the spermine complex with d(CGGGGGGTGCAGGGGG-GC)·d(GCCCCCCTGCACCCCCCG). The complex is viewed looking into the major groove. A 'U-shaped' spermine conformer is associated with a three-guanosine tract proximal to the d(TGCA)·d(TGCA) central sequence of the duplex. The spermine is depicted in bold and the three-guanosine tract is also in bold, relative to the remainder of the DNA duplex.

terminal amines and a rise of ~3.4 Å between base pairs of a typical B-DNA duplex). However, fully solvated computer simulations of spermine suggest that the all *trans* conformation is unfavorable because it exposes the hydrophobic parts of the spermine ion to water. This produces a driving force for the formation of a 'square planar' conformation. If this conformation is largely retained when the spermine is bound to poly-guanosine tracts of DNA, this might explain the requirement for only three contiguous guanines for efficient spermine binding.

The representation of how such an interaction might occur (Fig. 7) is provided only to show the relative sizes of the three-guanosine tract and the 'square planar' spermine conformer. It is not intended to show a specific binding orientation for spermine with the three-guanosine tract. It is clear that the spermine–DNA interaction is highly dynamic and includes general electrostatic interactions between charges on the DNA backbone and the spermine. However, it is possible that non-sequence-specific interactions do not fully describe the spermine association with DNA. As overtones to the general ionic association, there may be a number of more specific complexes that might modulate specific biophysical effects on the DNA, such as conformational transitions, bending and condensation. The complete elucidation of these sequence-selective binding modes may be a key to understanding these biophysical effects and, in turn, establishing the basis for the biological roles of the spermine–DNA interaction.

## ACKNOWLEDGEMENTS

Useful discussions, facilitated by NATO grant CRG 970290, were held with Dr Alison Rodger (University of Warwick, UK) and Dr Ian Blagbrough (University of Bath, UK). This work was supported by NIH grant CA64299-01 (I.S.H.).

## REFERENCES

1. Tabor,C.W. and Tabor,H. (1984) *Annu. Rev. Biochem.*, **53**, 749–790.
2. Goldemberg,S.H. and Algranati,I.D. (eds) (1989) *The Biology and Chemistry of Polyamines*, ICSU Symposium Series, Vol. 12.
3. McCann,P.P., Pegg,A.E. and Sjoerdsma,A. (1987) *Inhibition of Polyamine Metabolism*. Academic Press, Orlando, FL.
4. Poulin,R., Wechter,R.S. and Pegg,A.E. (1991) *J. Biol. Chem.*, **266**, 6142–6151.
5. Bergeron,R.J., Weimar,W.R., Muller,R., Zimmerman,C.O., McCosar,B.H., Yao,H. and Smith,R.E. (1998) *J. Med. Chem.*, **41**, 3901–3908.
6. Koza,R.A. and Herbst,E.J. (1992) *Biochem. J.*, **281**, 87–93.
7. Basu,H.S., Smirnov,I.V., Peng,H.F., Tiffany,K. and Jackson,V. (1997) *Eur. J. Biochem.*, **243**, 247–258.
8. Rodger,A., Taylor,S., Adlam,G., Blagbrough,I.S. and Haworth,I.S. (1995) *Bioorg. Med. Chem.*, **3**, 861–872.
9. Oller,A.R., Vanden Broek,W., Conrad,M. and Topal,M. (1991) *Biochemistry*, **30**, 2543–2549.
10. Thomas,T. and Kiang,D.T. (1988) *Nucleic Acids Res.*, **16**, 4705–4720.
11. Panagiotidis,C.A., Artandi,S., Calame,K. and Silverstein,S.J. (1995) *Nucleic Acids Res.*, **23**, 1800–1809.
12. Wei,T.-F., Bujalowski,W. and Lohman,T.M. (1992) *Biochemistry*, **31**, 6166–6174.
13. Ruiz-Herrera,J., Ruiz-Medrano,R. and Dominguez,A. (1995) *FEBS Lett.*, **357**, 192–196.
14. Garriga,P., Garcia-Quintana,D., Sagi,J. and Manyosa,J. (1993) *Biochemistry*, **32**, 1067–1071.
15. Musso,M., Thomas,T., Shirahata,A., Sigal,L.H., Van Dyke,M.W. and Thomas,T.J. (1997) *Biochemistry*, **36**, 1441–1449.
16. Musso,M. and Van Dyke,M.W. (1995) *Nucleic Acids Res.*, **23**, 2320–2327.
17. Hampel,K.J., Crosson,P. and Lee,J.S. (1991) *Biochemistry*, **30**, 4455–4459.
18. Thomas,T.J., Gunnia,U.B. and Thomas,T. (1991) *J. Biol. Chem.*, **266**, 6137–6141.
19. Basu,H.S., Schwietert,H.C.A., Feuerstein,B.G. and Marton,L.J. (1990) *Biochem J.*, **269**, 329–334.
20. Behe,M. and Felsenfeld,G. (1981) *Proc. Natl Acad. Sci. USA*, **78**, 1619–1623.
21. Flock,S., Labarbe,R. and Houssier,C. (1996) *Biophys. J.*, **70**, 1456–1465.
22. Smirnov,I.V., Dimitrov,S.I. and Makarov,V.L. (1988) *J. Biomol. Struct. Dyn.*, **5**, 1149–1161.
23. Hougaard,D.M., Bolund,L., Fujiwara,K. and Larsson,L.-I. (1987) *Eur. J. Cell Biol.*, **44**, 151–155.
24. Wilson,R.W. and Bloomfield,V.A. (1979) *Biochemistry*, **18**, 2192–2196.
25. Bloomfield,V.A. (1997) *Biopolymers*, **44**, 269–282.
26. Haworth,I.S., Rodger,A. and Richards,W.G. (1991) *Proc. R. Soc. Lond. B*, **244**, 107–116.
27. Feuerstein,B.G., Pattabiraman,N. and Marton,L.J. (1990) *Nucleic Acids Res.*, **18**, 1271–1282.
28. Feuerstein,B.G., Pattabiraman,N. and Marton,L.J. (1989) *Nucleic Acids Res.*, **17**, 6883–6892.
29. Zakrzewska,K. and Pullman,B. (1986) *Biopolymers*, **25**, 375–392.
30. Yuki,M., Grukhin,V., Lee,C.-S. and Haworth,I.S. (1996) *Arch. Biochem. Biophys.*, **325**, 39–46.
31. Schmid,N. and Behr,J.-P. (1991) *Biochemistry*, **30**, 4357–4361.
32. Marquet,R. and Houssier,C. (1988) *J. Biomol. Struct. Dyn.*, **6**, 235–246.
33. Wemmer,D.E., Srivenugopal,K.S., Reid,B.R. and Morris,D.R. (1985) *J. Mol. Biol.*, **185**, 457–459.
34. Padmanabhan,S., Brushaber,V.M., Anderson,C.F. and Record,M.T.,Jr (1991) *Biochemistry*, **30**, 7550–7559.
35. Tari,L.W. and Secco,A.S. (1995) *Nucleic Acids Res.*, **23**, 2065–2073.
36. Drew,H.R. and Dickerson,R.E. (1981) *J. Mol. Biol.*, **151**, 535–556.
37. Jain,S., Zon,G. and Sundaralingam,M. (1989) *Biochemistry*, **28**, 2360–2364.
38. Verdaguer,N., Aymami,J., Fernández-Forner,D., Fita,I., Coll,M., Huynh-Dinh,T., Igolen,J. and Subirana,J.A. (1991) *J. Mol. Biol.*, **221**, 623–635.
39. Tippin,D.B. and Sundaralingam,M. (1997) *J. Mol. Biol.*, **267**, 1171–1185.

40. Bancroft,D., Williams,L.D., Rich,A. and Egli,M. (1994) *Biochemistry*, **33**, 1073–1086.
41. Williams,L.D., Fredrick,C.A., Ughetto,G. and Rich,A. (1990) *Nucleic Acids Res.*, **18**, 5533–5541.
42. Haworth,I.S., Lee,C.-S., Yuki,M. and Gibson,N.W. (1993) *Biochemistry*, **32**, 12857–12863.
43. Lee,C.-S., Hartley,J.A., Berardini,M., Butler,J., Siegel,D., Ross,D. and Gibson,N.W. (1992) *Biochemistry*, **31**, 3019–3025.
44. Hartley,J.A., Berardini,M., Ponti,M., Gibson,N.W., Thompson,A.S., Thurston,D.E., Hoey,B.M. and Butler,J. (1991) *Biochemistry*, **30**, 11719–11724.
45. Pearlman,D.A., Case,D.A., Caldwell,J.C., Seibel,G.L., Singh,U.C., Weiner,P. and Kollman,P.A. (1991) *AMBER 4.0*. University of California, San Francisco, CA.
46. Molecular Simulations Inc. (1994) *QUANTA Version 4.0*. Molecular Simulations Inc., Burlington, MA.
47. Braunlin,W.H., Strick,T.J. and Record,M.T.,Jr (1982) *Biopolymers*, **21**, 1301–1314.
48. Olmsted,M.C., Anderson,C.F. and Record,M.T.,Jr (1989) *Proc. Natl Acad. Sci. USA*, **86**, 7766–7770.
49. Todd,A.K., Adams,A., Thorpe,J.H., Denny,W.A., Wakelin,L.P.G. and Cardin,C.J. (1999) *J. Med. Chem.*, **42**, 536–540.