Fourier Transform Raman Study of the Structural Specificities on the Interaction between DNA and Biogenic Polyamines

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ABSTRACT Biogenic polyamines putrescine, spermidine, and spermine are essential molecules for proliferation in all living organisms. Direct interaction of polyamines with nucleic acids has been proposed in the past based on a series of experimental evidences, such as precipitation, thermal denaturation, or protection. However, binding between polyamines and nucleic acids is not clearly explained. Several interaction models have also been proposed, although they do not always agree with one another. In the present work, we make use of the Raman spectroscopy to extend our knowledge about polyamine-DNA interaction. Raman spectra of highly polymerized calf-thymus DNA at different polyamine concentrations, ranging from 1 to 50 mM, have been studied for putrescine, spermidine, and spermine. Both natural and heavy water were used as solvents. Difference Raman spectra have been computed by subtracting the sum of the separated component spectra from the experimental spectra of the complexes. The analysis of the Raman data has supported the existence of structural specificities in the interactions, at least under our experimental conditions. These specificities lead to preferential bindings through the DNA minor groove for putrescine and spermidine, whereas spermine binds by the major groove. On the other hand, spermine and spermidine present interstrand interactions, whereas putrescine presents intrastrand interactions in addition to exo-groove interactions by phosphate moieties.

INTRODUCTION

Ornithine-derived polyamines spermine, spermidine, and putrescine (Fig. 1) are biogenic low molecular weight organic polycations that are essential for all living cells, regulating the rate and fidelity of macromolecular synthesis, cell proliferation/death equilibrium, cell differentiation, etc. (Tabor and Tabor, 1984; Pegg, 1988; Heby and Persson, 1990; Janne et al., 1991; Hayashi et al., 1996). It is well known that polyamines are essential molecules for optimal conformation of RNAs, and consequently they play an important role in protein translation (McMurry and Algranati, 1986). Intracellular concentrations of spermidine or spermine have been demonstrated to be critical for deciding between cell cycle progression and apoptosis (Ha et al., 1997). Direct interactions between the polyamines and nucleic acids, both RNA and DNA, were proposed to explain some of these biological functions (Cohen, 1998). Recently, the efficiency of the polyamines and polyamine analogues on the double-stranded (ds) DNA protection from oxidative breaking and radiation has been suggested (Ha et al., 1998a,b). However, the question about how they bind to nucleic acids has not been clearly established.

Early evidence for the effects of polyamines on dsDNA were related to precipitation and thermal denaturation (Tabor and Tabor, 1984). A first molecular interpretation of these phenomena came from crystallographic studies on different polyamine salts, mainly spermine. On the basis of such studies, different cross-linked models by the dsDNA minor groove were proposed (Suwalsky et al., 1969; Liquori et al., 1967; Tsuboi, 1964). These models involved electrostatic interactions between the polyamine amino protonated groups and the negatively charged dsDNA phosphate moieties; however, direct interactions with the dsDNA bases were discarded. Further studies on polyamine analogues showed the existence of structural specificities for dsDNA-polyamine complexation (Thomas and Bloomfield, 1984) in addition to the electrostatic effects. Different theoretical works (Pattabiraman et al., 1984; Feuerstein et al., 1986) agreed with this hypothesis; those works placed the spermine molecule interacting with the dsDNA bases across the major groove. On the other hand, crystals containing spermine/oligodeoxynucleotides were synthesized (Drew and Dickerson, 1981; Jain et al., 1989; Williams et al., 1990), and the subsequent studies by x-ray diffraction showed polyamine molecules were located along the dsDNA major groove, thus allowing for interactions with the bases. Most of these crystals contained B-form DNA oligomers, which proved the stabilization effects that polyamines have on dsDNA. Nevertheless, some controversy exists concerning the specific binding sites when polyamines interact with highly polymerized dsDNA in solution (Marquet and Houssier, 1988; Feuerstein et al., 1990; Ha et al., 1998a,b). As an example, NMR studies on DNA solutions indicated that polyamine-dsDNA interaction is highly localized (Andreasson et al., 1993). Summarizing, although some authors emphasize the electrostatic effects (Braunlin et al., 1982; Raspaud et al., 1998), it seems clear that this model alone cannot fully explain polyamine-dsDNA interaction (Basu et

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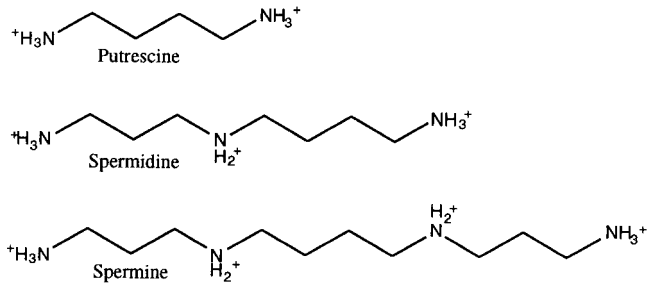

FIGURE 1 Chemical structures of the three ornithine-derived polyamines.

al., 1990; Pelta et al., 1996; Esposito et al., 1997); thus, the structural specificities have to play a significant role.

From a molecular point of view, improvement of the technical support to study structural changes and specificity during the polyamine-nucleic acid interaction becomes important to thoroughly understand the physiological roles of polyamines and to discriminate between direct polyamine-nucleic acid interactions and secondary effects of polyamine metabolism on nucleic acid conformation and dynamics. Among the different structural techniques available, vibrational spectroscopy allows for analyzing interacting systems at an atomic level, giving detailed and extensive structural information. Fourier transform spectroscopy has drastically improved the classical vibrational technique performance, largely by increasing sensitivity and scan speed; in addition, the Nd-YAG laser, usually employed as Fourier transform (FT)-Raman excitation radiation, allows for using higher laser powers without sample damaging, thus augmenting the spectral gain. In the present work, we have studied the interaction between highly polymerized dsDNA and the ornithine-derived polyamines by FT-Raman spectroscopy to achieve a deeper understanding of the polyamine-dsDNA interaction in solution. The results have been interpreted in terms of specific binding sites, which could exist in addition to purely electrostatic effects.

MATERIALS AND METHODS

Materials

Highly polymerized calf-thymus DNA sodium salt (6.2% sodium content; 13.0% H_2O content) and polyamines (spermine, spermidine, and putrescine) as poly-hydrochlorides were purchased from Sigma Chemical Co. (St. Louis, MO). To check the protein content of DNA, ultraviolet absorbance at 260 and 280 nm was measured. The A_{260}/A_{280} ratio was 1.75, indicating a low protein content.

Sample preparation

Solutions at different polyamine concentrations were prepared. To preserve physiological pH and avoid DNA-strand splitting effects, 20 mM TRIS buffer, 200 mM sodium chloride, was always used as the solvent. Final pH was adjusted to 7.5 by using hydrogen chloride. Polyamine-dsDNA complexes were prepared by adding dsDNA to the polyamine solutions, up to 2% final dsDNA concentration (0.06 M, phosphate). The mixtures were slowly stirred for 48 h at 4°C to ensure the formation of homogeneous solutions and were subsequently kept at the same temperature until recording the spectra (2 days maximum). Solutions using both H_2O and D_2O (99% D; Aldrich, Milwaukee, WI) as solvent were prepared for the three ornithine-derived polyamines.

Raman measurements

FT-Raman spectra were recorded in a Bruker Equinox 55 Fourier transform spectrometer supplied with a Raman module. Spectra were obtained at a spectral resolution of 2 cm^{-1}, using excitation radiation wave number at 1064 nm from a Nd-YAG laser working at 500 mW. We used a standard quartz cell for liquids (1-cm section) where approximately 1 ml of the solutions were placed. Back-scattering collection of the Raman radiation was performed using a mirror behind the cell, and a minimum of 2000 scans were accumulated in all cases to enhance the signal-to-noise ratios. Individual scans were examined by the recording routine, being automatically discarded when the mean intensity deviations were greater than 10% over the full interpherogram length. This procedure prevents artifacts in the resulting spectra before averaging and was performed at least with two independently prepared samples for each concentration. To discuss the Raman peaks, the following criteria were adopted: 1) peaks must appear with the same wave number and intensity in spectra of independent samples; 2) peaks must exhibit an intensity clearly greater than the experimental noise level; and 3) peaks should be previously reported and assigned in the literature. Spectral treatment was performed by using the Bruker OPUS 227 spectroscopic software. Raman spectra were normalized between 600 and 1800 cm^{-1} to preserve them from baseline deviations and were subsequently corrected by subtracting the spectrum of a buffer solution prepared at identical experimental settings.

Difference spectra were computed by subtracting the sum of the separately measured dsDNA and polyamine spectra from the corresponding experimental spectra of the complexes, each previously normalized. This procedure has two main consequences: to prevent baseline effects on the difference spectra and to establish a suitable criterion for measuring the positive and negative difference bands. We have employed the following criteria to discuss the Raman difference spectra: 1) the difference bands should exhibit an intensity ratio at least twice the noise level; 2) the difference features must be structurally interpretable, and 3) the difference features must be reproducible for independently prepared samples. In addition, conclusions should be confirmed by both H_2O and D_2O solutions.

RESULTS AND DISCUSSION

Analysis of spectroscopic data

The FT-Raman spectra of calf-thymus DNA, the three ornithine-derived polyamines, and DNA-polyamine complexes, at identical experimental settings (buffer concentration and pH) are displayed in Figs. 2 and 3, which correspond to natural water and heavy water, respectively. They show a dsDNA-polyamine spectrum for each polyamine as an example. The relevant wave numbers for the complete range of concentrations studied are listed in Tables 1 and 2. As is known (Gosule and Schellman, 1976), polycations induce condensation and precipitation of the negatively charged DNA strands; this effect is further enhanced when the positive charge of the counterion is increased. The three ornithine-derived polyamines are polycations at physiological pH; however, dsDNA precipitation

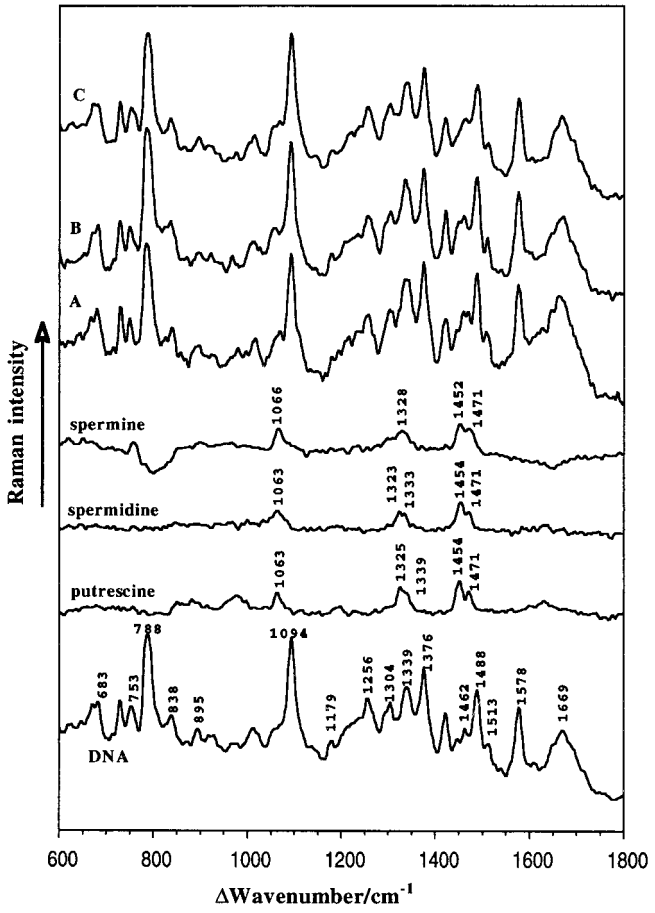

FIGURE 2 Raman spectra of calf-thymus DNA (60 mM, phosphate), the three ornithine-derived polyamines (60 mM), and some DNA-polyamine complexes in natural water. (*A*) DNA-putrescine, 25 mM; (*B*) DNA-spermidine, 25 mM; (*C*) DNA-spermine, 5 mM.

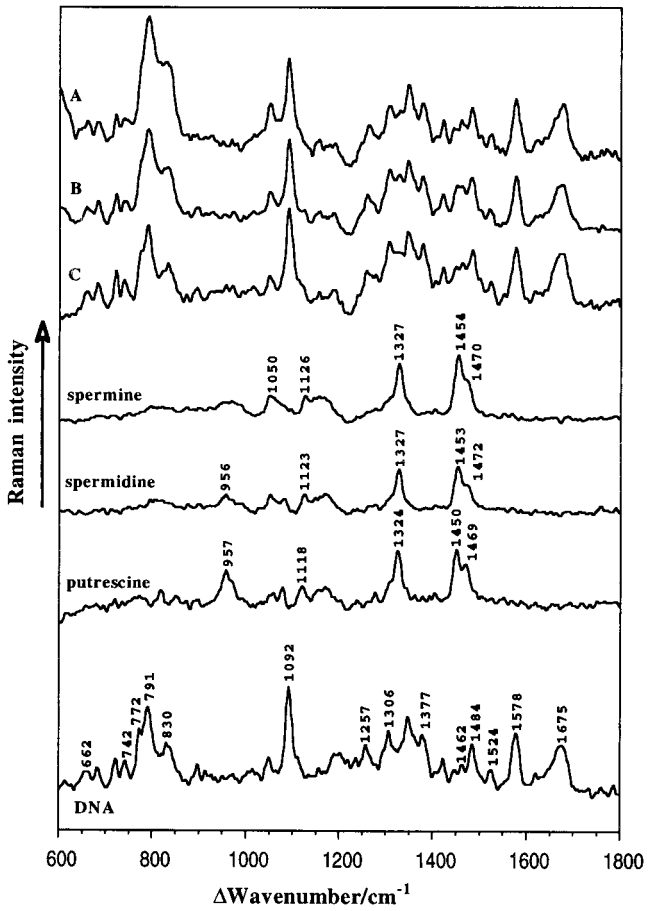

FIGURE 3 Raman spectra of calf-thymus DNA (60 mM, phosphate) , the three ornithine-derived polyamines (60 mM), and some DNA-polyamine complexes in heavy water. (*A*) DNA-putrescine, 25 mM; (*B*) DNA-spermidine, 25 mM; (*C*) DNA-spermine, 5 mM.

with spermine (charge +4) occurs at a lower concentration than for spermidine (+3) and is not observed with putrescine (+2), in agreement with the fact that divalent ions do not induce dsDNA precipitation in aqueous solutions (Rouzina and Bloomfield, 1998). This fact is why the different molar ratios were selected. Because dsDNA precipitation is observed in our experimental conditions when spermine concentrations are greater than ~10 mM, spermine solutions ranged between 1 and 7.5 mM, which are physiological concentrations. In fact, millimolar concentrations of polyamines have been found in the nucleus of eukaryotic cells (Sarhan and Seiler, 1989). In the case of spermidine and putrescine, solutions at concentrations up to 50 mM were studied without observing condensation phenomena. This behavior agrees with recent studies on oligodeoxyribonucleotides (Thomas et al., 1997), which showed that putrescine and spermidine do not induce changes in the circular dichroism spectra of the oligomers. The FT-Raman spectra of the complexes, however, show wave number shifts for all the solutions, indicating that interactions are present with or without condensation.

As mentioned, we have obtained Raman difference spectra between the experimental spectra of the dsDNA-polyamine complexes and the computed sum of the separated components spectra. Differences have been computed for the highest polyamine concentrations studied in each case. Figs. 4 and 5 show Raman difference spectra of dsDNA-polyamine complexes in H_2O and D_2O, respectively; as an example, we have added the normalized spectrum of the complex and the sum spectrum of the separated constituents for putrescine.

Raman spectra of solutions in H_2O

Vibrational spectra of dsDNA have been extensively studied in recent years, although not all the bands have been undoubtedly assigned up to now. In the case of Raman spectra, bands mainly arise from both base and phosphate vibrations, together with some deoxyribose contributions (Spiro, 1987; Thomas and Tsuboi, 1993). The region between 1700 and 1200 cm^{-1} is clearly dominated by base

TABLE 1 Relevant wave numbers (in cm^{-1}) measured in the Raman spectra of polyamine-DNA (60 mM phosphate) complexes at different polyamine concentrations solved in natural water

	Putrescine			Spermidine			Spermine			
DNA	5 mM	25 mM	50 mM	5 mM	25 mM	50 mM	1 mM	5 mM	7.5 mM	Assignments
		1675	1670							
1669	1667	1664	1662	1670	1671		1669	1669	1669	Thynine (O2)
1578	1577	1576	1577	1576	1576	1576	1578	1578	1578	Purine stretching
1513	1510	1509	1510	1509	1510	1510	1509	1511	1511	Adenine
1488	1488	1488	1488	1488	1488	1488	1489	1490	1490	Guanine (N7)
1462	1461	1460	1459	1462	1461		1462	(1463)		Deoxyribose
1376	1375	1375	1375	1375	1374	1374	1375	1375	1375	Thymine (CH_3), purine
1339	1338	(1338)	1337	1337	1336	1335	1339	1339	1339	Purine stretching
1304	1303	1301	1302	1303	1304	1306	1303	1304	1304	Adenine
1256	1254	1254	1255	1259	1255	1255	1257	1257	1257	Adenine, cytosine
1179	1175	1178	1176	1175	1178	1178			1177	Thymine, cytosine
1094	1093	1093	1093	1093	1092	1092	1094	1094	1094	Phosphodioxy stretching
895	896	894	893	893	(895)	894	896	897	897	Deoxyribose
838	837	(839)	833	837	836	835	835	836	838	Phosphodiester stretching
788	785	786	788	788	786	788	788	788	788	Phosphodiester stretching
753	751	750	750	751	750	750	753	753	753	Thymine
683	680	680	681	683	682	680	678	681	681	Guanine

Wave numbers in parentheses are subject to major errors. Assignments have been taken from the literature; relevant references are given in the Discussion.

vibrations, which involves the stretching modes of the aromatic rings, the carbon-oxygen double bond stretching, and some methyl bending vibrations. Methylene scissoring modes of the deoxyribose units are also observed around 1400–1450 cm^{-1}. The broad band that can be observed in all of the Raman spectra around 1670 cm^{-1} corresponds to the C═O stretching modes, and it has been largely assigned to the C2═O2 bond of thymine residues (Spiro, 1987; Puppels et al., 1994). The thymine O2 atoms are a putative site of interaction of dsDNA at the minor groove because they are not involved in Watson-Crick hydrogen bonds. After dsDNA/spermidine or putrescine complexation, this band shifts by several cm^{-1}; however, it does not shift when adding spermine at different molar ratios. The opposite behavior is observed in the dsDNA band at 1488 cm^{-1}, an aromatic ring stretching vibrations in which the N7 atom of guanine, located at the major groove, has the largest vibrational amplitude (Spiro, 1987; Thomas and Tsuboi, 1993;

TABLE 2 Relevant wave numbers (in cm^{-1}) measured in the Raman spectra of polyamine-DNA (60 mM phosphate) complexes at different polyamine concentrations solved in heavy water

	Putrescine			Spermidine			Spermine			
DNA	5 mM	25 mM	50 mM	5 mM	25 mM	50 mM	1 mM	5 mM	7.5 mM	Assignments
1675	1676	1677	1675	1677	1676	1676	1678	1677	1676	Thymine (O2)
	1667	1670		1669	1667					
1578	1575	1576	1576	1577	1577	1577	1577	1576	1577	Purine stretching
1524	1524	1523	1520	1521	(1520)	1521	1522	1521	1522	Adenine
1484	1483	1484	1484	1484	1483	(1481)	1485	1482	1483	Guanine (N7)
1462	1464	1462	1462	1461	1460		1462	1460	1459	Deoxyribose
1377	1377	1377	1377	1377	1378	1378	1378	1378	1378	Thymine (CH_3), purine
1306	1306	1305	1306	1304	1306	1307	1307	1307	1305	Adenine
1257	1259	1257	1259	1259	1258	1261	1260	1262	1258	Cytosine (amide III)
1092	1091	1091	1091	1092	1091	1090	1091	1091	1091	Phosphodioxy stretching
830	831	832	831	832	832	831	831	(828)	832	Phosphodiester stretching
791	790	788	788	790	790	789	791	790	790	Phosphodiester stretching
772	775	776	(775)	774			(774)		(775)	Cytosine
742	740	738	738	739	739	739	741	738	741	Thymine
662						662				
	660	659	660	659	659		663	660	661	Thymine
657						659				

Wave numbers in parentheses are subject to major errors. Assignments have been taken from the literature; relevant references are given in the Discussion.

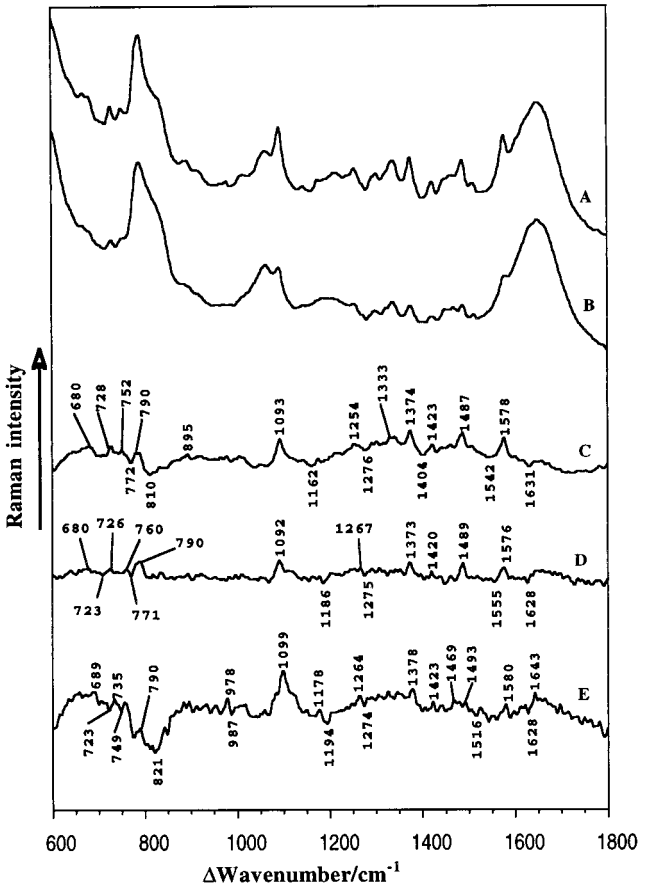

FIGURE 4 Raman and difference spectra from solutions in natural water. (*A*) Spectrum of a solution of 60 mM DNA (phosphate) and 50 mM putrescine; (*B*) The spectrum sum of separated constituents; (*C*) The difference spectrum; (*D*) The difference spectrum (four times amplified) for a solution of 60 mM DNA (phosphate) and 50 mM spermidine; (*E*) The difference spectrum (four times amplified) for a solution of 60 mM DNA (phosphate) and 7.5 mM spermine.

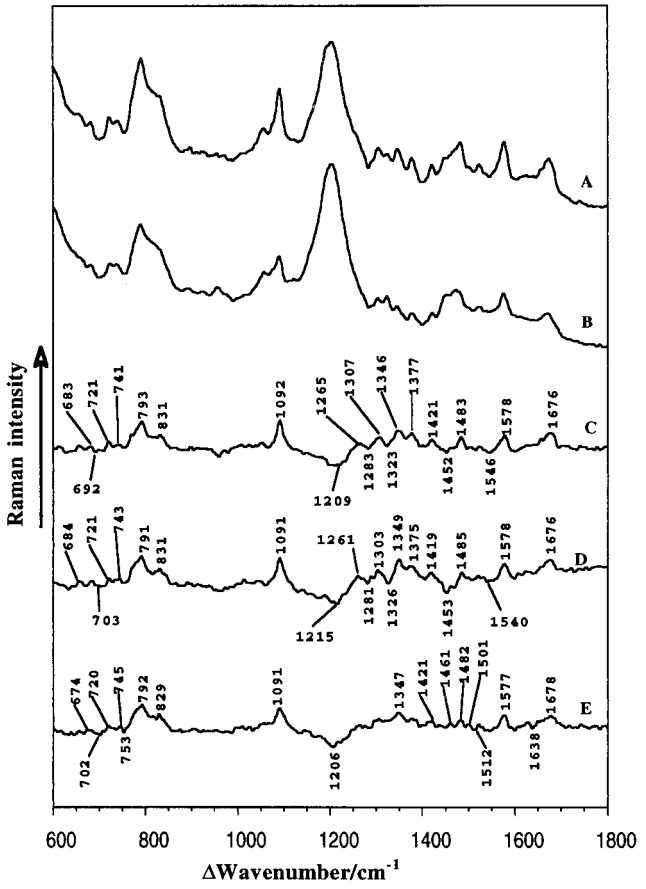

FIGURE 5 Raman and difference spectra from solutions in heavy water. (*A*) Spectrum of a solution of 60 mM DNA (phosphate) and 50 mM putrescine; (*B*) The spectrum sum of separated constituents; (*C*) The difference spectrum; (*D*) The difference spectrum for a solution of 60 mM DNA (phosphate) and 50 mM spermidine; (*E*) The difference spectrum for a solution of 60 mM DNA (phosphate) and 7.5 mM spermine.

Puppels et al., 1994; Krafft et al., 1998). It shifts upwards by 1–2 cm^{-1} in the Raman spectra of spermine-dsDNA complexes, although remaining unaltered for all the putrescine and spermidine solutions. These results are supported by the Raman difference spectra (Fig. 4). For spermine, the positive bands at 1469 and 1493 cm^{-1} have been assigned to an interaction with the guanine-N7 reactive site, whereas the positive feature at 1643 cm^{-1} and the negative feature at 1628 cm^{-1} have been assigned to interactions with C═O groups. In the Raman difference spectrum of spermidine, the positive band at 1489 cm^{-1} does not appreciably deviate with respect to the experimental wave number of 1488 cm^{-1}. This fact has also been observed in the difference spectrum of putrescine and is consistent with the behavior of this Raman band when the putrescine or spermidine concentrations vary (Table 1).

The Raman difference spectrum of putrescine (Fig. 4) shows a general increasing of the bands (hypochromism) appearing between 1700 and 1200 cm^{-1}. This fact has been largely attributed to a base unstacking (Erfurth and Peticolas, 1975; Peticolas, 1975; Benevides et al., 1991) as a consequence of the interaction. The difference spectrum of spermidine also exhibits Raman hypochromism, although the intensity increase is much lower than for putrescine, although this is not observed as a general effect in the Raman difference spectrum of spermine. The dsDNA band at 1513 cm^{-1} shifts downward in the spectra of all the complexes studied, with slightly greater differences corresponding to the solutions at the lower polyamine-dsDNA molar ratios. This band has been assigned to an adenine carbon-carbon stretching vibration (Spiro, 1987; Thomas and Tsuboi, 1993). The strong band measured in the Raman spectrum of dsDNA at 1578 cm^{-1} has also been assigned to a purine vibration (Puppels et al., 1994; Krafft et al., 1998). However, its behavior discriminates spermine, for which this band does not shift at any molar ratio; 1–2-cm^{-1} shifts have been observed for the other two polyamines. Within

this spectral region, a group of three bands measured between 1470 and 1420 cm^{-1} in the Raman spectrum of dsDNA has been assigned to the deoxyribose moieties (Thomas and Tsuboi, 1993; Carmona and Molina, 1990; Overman et al., 1998). They correspond to methylene bending modes, although they should also have some contributions from adenine vibrations (Lord and Thomas, 1967; Spiro, 1987). Interpretation of their wave number shifts is, nevertheless, troublesome for solutions at the highest polyamine concentrations, because they also contain methylene groups. However, a positive difference band at 1378 cm^{-1} in the difference spectrum of spermine indicates hydrophobic contacts with the methyl groups of thymine residues (Krafft et al., 1998). Thymine-methyl groups are located at the dsDNA major groove. The Raman spectra of both spermidine and putrescine show a pair of bands around 1471 and 1454 cm^{-1}; the polyamine-dsDNA spectra at the higher polyamine concentrations show a peak at ~1450 cm^{-1}, whereas no maximum is observed at 1471 cm^{-1} because of its lower intensity. Both wave numbers can be clearly assigned to the polyamine methylene scissoring vibrations (Bellamy, 1981), so these results support the existence of hydrophobic interactions between the polyamine molecules and dsDNA.

Further evidence of polyamine-dsDNA interactions by the bases can be obtained from the DNA bands at 1339 cm^{-1} (adenine and guanine) (Spiro, 1987; Neault et al., 1996), 1304 cm^{-1} (adenine) (Thomas and Tsuboi, 1993; Overman et al., 1998), 1256 cm^{-1} (adenine and cytosine) (Lord and Thomas, 1967; Overman et al., 1998), and 1179 cm^{-1} (thymine and cytosine) (Puppels et al., 1994; Overman et al., 1998). All of them shift up to 4 cm^{-1} in the spectra of the complexes. The band at 1339 cm^{-1} corresponds to a nonresolved doublet whose components could have different characters. In addition, it is overlapped with two polyamine Raman bands that can be assigned to methyl, methylene, and/or ammonium vibrations. The spectra in D_2O will allow assigning them correctly, which is a prerequisite to interpreting their behaviors upon DNA complexation.

In the Raman difference spectrum of spermine, features can be measured at 1274 (−), 1264 (+), 1194 (−), and 1178 (+) cm^{-1}, which have been largely assigned to pyrimidine vibrations. The interaction of spermidine with pyrimidine residues is supported by the difference bands at 1275 (−), 1267 (+), and 1186 (−) cm^{-1}. For putrescine, only difference bands at 1276 (−) and 1254 (+) cm^{-1} have been measured, which have been assigned to a cytosine vibration. In the region of ring breathing vibrations, the positive difference band at 689 cm^{-1} indicates interaction of spermine with guanine residues, whereas the positive feature at 735 cm^{-1} and the negative one at 723 cm^{-1} have been assigned to an adenine vibration (Spiro, 1987; Thomas and Wang, 1988; Cao et al., 1995; Overman et al., 1998). The positive bands at 728 cm^{-1} for putrescine and 726 cm^{-1} for spermidine have also been assigned to an adenine vibration, whereas those at 752 cm^{-1} and 760 cm^{-1}, respectively, indicate interaction with thymine residues.

The Raman spectrum of dsDNA between 1100 and 800 cm^{-1} is dominated by two intense bands, namely, at 1094 and 788 cm^{-1}, which have been assigned to the symmetrical stretching vibrations of the phosphodioxy (PO_2^-) and phosphodiester (O-P-O) moieties, respectively (Spiro, 1987; Thomas and Wang, 1988; Cao et al., 1995). In addition, a second O-P-O peak is measured at 838 cm^{-1} (Ghomi et al., 1990), which is considered as one of the DNA conformational marker bands. The Raman difference spectra from solutions in H_2O (Fig. 4) provide clear evidence for interaction between dsDNA and the polyamines by the negatively charged phosphodioxy groups, as deduced from the negative features appearing ~1094 cm^{-1} for the three polyamines. A second common feature is the negative band at 790 cm^{-1}, which is assigned to the O-P-O stretching vibrations. This fact indicates that phosphodiester valence electrons are less attached to the related nuclei after polyamine complexation, thus confirming that polyamine-DNA interactions involve changes on the DNA backbone.

The O-P-O marker band at 838 cm^{-1} needs to be discussed separately. This band is very sensitive to both conformation and base sequence, shifting by 10 cm^{-1} from a CG sequence to an AT sequence of B-DNA (Thomas and Wang, 1988). Consequently, for native DNA, it usually appears as a broad band that cannot be split by deconvolution procedures. For the polyamine-dsDNA Raman spectra, some deviations are observed with respect to the wave number measured for calf-thymus DNA. However, they do not lead to conformational transitions A↔ B↔ Z in any case; in our opinion, the observed shifts are due to small conformational distortions originated by the molecular interaction. Other weak Raman bands in this spectral region have been assigned to stretching vibrations of the deoxyribose rings, namely, at 1012 and 895 cm^{-1} for calf-thymus DNA (Spiro, 1987; Thomas and Tsuboi, 1993). Although they are both weak bands, their shifts upon polyamine complexation indicate contribution of the sugar moieties in the interaction. Finally, between 650 and 800 cm^{-1} the Raman spectrum of dsDNA shows some medium intensity bands that correspond to vibrational bending modes of the nucleic bases. Regarding the wave number shifts upon polyamine complexation, preferential link to guanine residues can be inferred for spermine, in agreement with the precedent discussion for the stretching modes of the bases, between 1700 and 1200 cm^{-1}. Shifts measured for putrescine-dsDNA and spermidine-dsDNA solutions involve purine and pyrimidine residues, although only the thymine band at 753-cm^{-1} shifts monotonically with respect to the polyamine concentration.

The observation of the NH_3^+ stretching and bending vibrations for any solute of a water solution is not an easy task because they lie close to the related O-H vibrations of the solvent molecules, ~3000 and 1700 cm^{-1}, respectively.

However, the NH_3^+ groups have a third type of vibrational mode, usually termed the rocking mode (Bellamy, 1981), which gives rise to one or two Raman bands around 1100 cm^{-1}. It has been observed at 1063 cm^{-1} for both spermidine and putrescine. At the higher polyamine concentrations, it goes to lower wave numbers, as a probe of the active participation of these groups in the interactions.

Raman spectra of solutions in D_2O

The Raman study of polyamine-dsDNA complexes was extended to solutions in heavy water. The FT-Raman spectra of the solutions in heavy water are displayed in Fig. 3, whereas the difference Raman spectra are displayed in Fig. 5. Relevant wave numbers have been summarized in Table 2. The hydrogen-deuterium exchange causes a general shift downward to the vibrational frequencies of both dsDNA and the polyamines. However, for a complex polymer such as dsDNA, we cannot expect an exact correlation between the vibrational wave numbers of deuterated and nondeuterated samples; in other words, close Raman bands can correspond to different normal modes, so some shifts to higher wave numbers could be observed upon deuteration, which would be impossible for a little molecule. Nevertheless, the close bands can usually be assigned to the same vibration on the basis of their major vibrational coordinate contributions. Consequently, and despite the lack of vibrational information concerning deuterated DNA, most of the Raman bands can be assigned on the basis of previously reported studies (Prescott et al., 1984; Krafft et al., 1998) and the proposed assignments for nondeuterated dsDNA (Spiro, 1987; Thomas and Wang, 1988; Thomas and Tsuboi, 1993). As the solutions in D_2O were stored at 4°C and used within 2 days after preparation, we assume that the acidic, nitrogen-attached hydrogens of the bases have been fully exchanged, whereas the nonacidic, carbon-attached hydrogens remain unsubstituted (Benevides and Thomas, 1985). This fact will give rise to small shifts for most of the Raman bands, because they largely correspond to base aromatic stretching, deoxyribose methylene bending, and phosphate stretching vibrations. We also assume all the NH_3^+ groups of the polyamine molecules are fully deuterated.

Raman difference spectra from solutions in D_2O are consistent with those obtained from solutions in H_2O. However, some deviations exist as can be observed in Fig. 5. In our opinion they are largely due to 1) solutions in D_2O being better scatterers than in H_2O, giving rise to more intense Raman spectra and 2) the lack of an exact correlation between both sets of normal coordinates, as already mentioned.

In the region between 1700 and 1200 cm^{-1} the most outstanding results are related to the broad carbonyl stretching band (mainly thymine) around 1675 cm^{-1} (Spiro, 1987) and to the intense purine aromatic stretching band at 1484 cm^{-1} (Krafft et al., 1998). The last wave number has been measured at 1488 cm^{-1} for natural calf-thymus DNA and is strongly related to the N7 atom of guanine, as already mentioned. We assume this assignment is also relevant for the 1484-cm^{-1} band of deuterated dsDNA. Raman spectra of both putrescine- and spermidine-dsDNA complexes in D_2O solutions confirm that interactions involve thymine O_2 atoms in the dsDNA minor groove. Negligible changes are observed for the band at 1484 cm^{-1} of deuterated DNA when putrescine is added. Identical behavior is observed for dsDNA-spermidine (5 mM). Greater spermidine concentrations originate wave number shifts up to -4 cm^{-1}; however, in our opinion this is the result of overlapping between the dsDNA band and those corresponding to the methylene bending vibrations of spermidine. Concerning spermine-dsDNA solutions, Raman spectra support the interaction through the guanine N7 sites at the dsDNA major groove. In addition, we would like to emphasize that small shifts upward have been observed for the carbonyl stretching band. This fact suggests that the spermine-dsDNA interaction could also involve the minor groove, especially at low polyamine concentrations. Interaction with the O6 atom of guanine could also be postulated as the band at 1675 cm^{-1} contains contributions from all the carbonyl groups of the DNA bases: guanine, cytosine, and thymine.

The Raman difference features measured in this spectral region for putrescine in D_2O can be well correlated with those measured for the H_2O solution. In addition, a clear hypochromic effect is also evident; this phenomenon decreases when augmenting the polyamine molecular size. Other Raman bands also establish differences between longer and shorter polyamines, such as the dsDNA band at 1524 cm^{-1}, mainly assigned to an adenine vibration (Spiro, 1987). For putrescine, the related shifts vary monotonically with the concentration, whereas for spermidine and spermine they are almost invariant with the polyamine concentration.

Concerning the deoxyribose methylene bending bands between 1470 and 1420 cm^{-1}, they behave similarly for both D_2O and H_2O solutions. All the polyamine-dsDNA complexes show appreciable shifts of the dsDNA band at 1257 cm^{-1}, which has been assigned to a cytosine amide III vibration (Krafft et al., 1998); other authors, however, propose it as having some contributions from the purine residues, largely adenine. This is a medium-intensity peak that does not appreciably shift upon deuteration, also behaving in a similar way in both natural and heavy water. The isotopic shifts measured for the bands corresponding to the phosphate moieties agree with those observed for the H_2O solutions. The phosphodioxy band at 1092 cm^{-1} slightly shifts upon spermine complexation, -1 cm^{-1} for all the concentrations studied, whereas the phosphodiester stretching band at 830 cm^{-1} exhibits changes in the three cases. The second O-P-O band, measured at 791 cm^{-1} for dsDNA in D_2O, also shifts downward for the three polyamines; the deviations seem to be influenced by both polyamine size and concentration, showing greater shifts when increasing

the concentration or decreasing the molecular size. The interaction with the phophodioxy groups is also supported by the positive feature observed for the PO_2^- stretching band at 1090 cm^{-1}. In addition, the three difference spectra also show a positive band centered at 791 cm^{-1}, which can be assigned to the phosphodiester stretching vibration.

Hydrogen-deuterium exchange allows us to identify some NH_3^+ bending and rocking vibrations in the Raman spectra of polyamine molecules. Comparing the spectra of H_2O with those of D_2O solutions, we confirm our previous hypothesis on the assignment of a NH_3^+ rocking vibration for the band at ~1065 cm^{-1} that appeared in the Raman spectra of the three ornithine-derived polyamines in H_2O. In addition, these spectra showed a doublet centered around 1328 cm^{-1} that changes to a single band when deuterated, thus indicating that one of the wave numbers has to be assigned to an NH_3^+ bending vibration. Polyamine bands that do not significantly alter their wave numbers upon deuteration can be assigned to methylene vibrations. This band shifts -3 cm^{-1} for putrescine and -1 cm^{-1} for spermidine. A little shoulder can be measured at ~1331 cm^{-1} for dsDNA-spermine 7.5 mM solution in D_2O, despite its low concentration; it corresponds to the methyl/methylene band of the polyamine measured at 1327 cm^{-1}. These data support the existence of hydrophobic interactions between dsDNA and the polyamines.

In the region between 650 and 800 cm^{-1} we would like to emphasize the observed shifts downward for the band at 772 cm^{-1}. It has been largely assigned to a cytosine vibration (Krafft et al., 1998), and deviations up to 3–4 cm^{-1} have been measured for the three polyamines studied. For solutions in H_2O, this band is hidden behind the intense phophodiester band at 788 cm^{-1}. The modifications observed for the rest of the bands in this region are related to the dsDNA band at 742 cm^{-1} and the doublet at 662 and 657 cm^{-1}. All of them shift downward by 3–4 cm^{-1} after polyamine complexation, and have been assigned to thymine vibrations.

Discussion of preferential binding sites

The results obtained from this Raman spectroscopic study can be discussed in terms of preferential sites of binding between DNA and the polyamines, which can be compared with interaction models proposed on the basis of different biochemical, physical, and chemical techniques. Raman data indicate interaction by the bases for the three polyamines, although they establish some structural-dependent differences. To propose some interaction models, the intramolecular distances have to be taken into account. For polyamines, we have assumed the all-*trans* structures as the starting point in the three cases. As predicted by theoretical calculations (Marques and Batista de Carvalho, 2000) this is the lowest-energy conformation for these polyamines. Intramolecular distances for dsDNA and polyamines can be approximately calculated using standard geometrical parameters (Hypercube, 1996). Thus, the N-N distance in an all-*trans* putrescine molecule is ~6.2 Å, as separated by a tetramethylene chain. For spermidine and spermine there are also two nitrogen atoms separated by a trimethylene chain of ~5.0 Å. These distances can be correlated with some dsDNA interatomic lengths between the binding sites led by the spectroscopic results.

Putrescine

Raman spectra of dsDNA-putrescine solutions indicate preferential binding by the DNA minor groove, involving both phosphodioxy and bases, together with some hydrophobic interactions. This hypothesis is supported by the guanine-N7 band at 1488 cm^{-1} and for the thymine methyl-bending band at 1377 cm^{-1}; both bands do not shift when putrescine concentration changes. Both sites lie at the dsDNA major groove. In addition, the carbonyl (thymine and cytosine, minor groove) band at 1669 cm^{-1} does monotonically shift upon putrescine complexation. Other features related with purine aromatic stretching bands (1513, 1339, and 1304 cm^{-1}) could be explained by putrescine-dsDNA interactions involving the purine-N3 sites at the minor groove, although the molecular size of putrescine could allow it to interact by both minor and major grooves. The measured difference features for the phosphodioxy Raman band at 1092 cm^{-1} in both natural and deuterated samples also indicate close contacts through the polyamine NH_3^+ groups.

It is known that the more reactive site at the major groove is the guanine-N7 atom, because it is free from strong steric hindrances and is not involved in the Watson-Crick hydrogen bonds. However, the distances from this atom to the nearest intrastrand phosphate groups are around 5.5 and 7.1 Å, which are not suitable for interacting with the two NH_3^+ groups of a putrescine molecule (6.2 Å). On the other hand, the main reactive sites at the minor groove are the O2 atom of thymine and the N3 atom of purine bases. Estimated lengths of 5.9 and 6.1 Å can be obtained for O2(thymine)-PO_2^- and N3(guanine, adenine)-PO_2^-, which correlate well with the putrescine N-N distance. We therefore propose putrescine-dsDNA complexation models where the polyamine NH_3^+ groups are in contact with a phosphate group and either a thymine-O2 or a guanine/adenine-N3, as displayed in Fig. 6, *A* and *B*, respectively. As can be seen in Fig. 6 *A*, this model allows for some hydrophobic interactions between the methylene moieties of putrescine and those of dsDNA (both skeletal and deoxyribose CH_2), in agreement with the Raman results. The significant shifts measured for the Raman band at 772 cm^{-1} of deuterated dsDNA indicate that cytosine residues are also involved in the putrescine-DNA complexation, which should occur through the cytosine-O2 site in the same way as for the thymine-O2 atoms. On the basis of the observed Raman

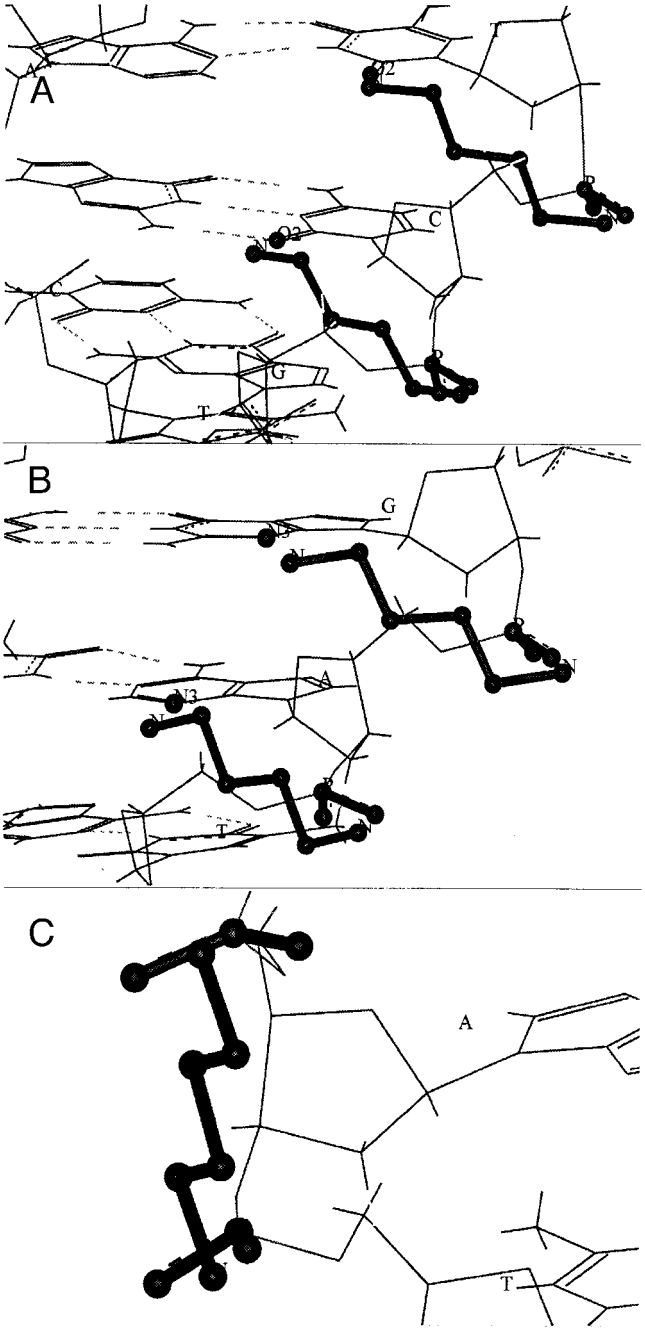

FIGURE 6 Preferential binding model proposed for putrescine-DNA complexation. (*A*) Phosphate and pyrimidine-O2 from the same strand of the minor groove; (*B*) Phosphate and purine-N3 from the same strand of the minor groove; (*C*) Phosphate-phosphate exo-groove.

difference features for phosphodioxy (PO_2^-) and phosphodiester (O-P-O) groups and the estimated distances for two adjacent phosphates, ~6.1 Å, we also propose a third complexation model shown in Fig. 6 *C*. This is an exo-groove interaction where the putrescine NH_3^+ group contacts two consecutive dsDNA phosphate moieties.

The binding models proposed for DNA-putrescine interaction can also be interpreted in relation to the Raman hypochromism observed in the difference spectra. As can be observed in Fig. 6, *A–C*, these models involve only intrastrand bindings. This fact is consistent with recent results about DNA protection from radiation-induced strand breakage by naturally occurring polyamines (Sy et al., 1999; Warters et al., 1999; Douki et al., 2000), which have demonstrated the ability of spermidine and spermine to radioprotect DNA, whereas putrescine is not able to. Intrastrand bindings cannot prevent DNA from strand breakage; in addition, putrescine molecules would have the effect of altering the interstrand hydrogen bond network at a high putrescine/DNA molecular ratio (as happens for the DNA 60 mM-putrescine 50 mM complexes), thus giving rise to the aforementioned hypochromism.

Spermidine

The molecular size of putrescine prevents it from interstrand interactions. However, the N-N distance in an all-*trans* spermidine molecule reaches ~11.2 Å, which would allow for interaction models involving two reactive sites located at different DNA strands. As mentioned above, the interstrand interactions could justify the radioprotection of dsDNA by spermidine.

Raman data for DNA-spermidine solutions indicate preferential binding by the DNA minor groove. As for putrescine, this can be largely inferred from the observed features for the DNA bands at 1669 and 1488 cm^{-1}, as previously discussed. The phosphodioxy Raman band is also subject to monotonical shift upon spermine complexation, and its intensity increases as deduced from the Raman difference spectrum at a spermidine concentration of 50 mM. Considering the interatomic distances at the minor groove, there are suitable values for the spermidine interaction involving the purine-N3 and pyrimidine-O2 atoms and a phosphate group from the same strand (~11.0–11.5 Å). However, these intrastrand interactions would need the polyamine to be adapted to the helix rotation; otherwise, strong steric hindrances could appear. Thus, as preferential models of binding we propose those displayed in Fig. 7, *A* and *B*, in which an outer NH_3^+ group of spermidine is near a dsDNA phosphate moiety; two interstrand binding possibilities could exist for the second NH_3^+ group: pyrimidine-O2 and purine-N3, whose distances to the PO_2^- groups are between 10.5 and 12.5 Å, respectively. These models are free from steric hindrances, and they are in good agreement with the Raman evidence. In addition, the N3-PO_2^- model, Fig. 7 *B*, allows for interaction between a thymine-O2 site and the inner spermidine NH_2^+ group, although this is only when the N3 atom corresponds to an adenine residue. Hydrophobic contacts with the methylene-C5′ can also occur. An alternative binding model involving two pyrimidine-O2 centers has been depicted in Fig. 7 *C*.

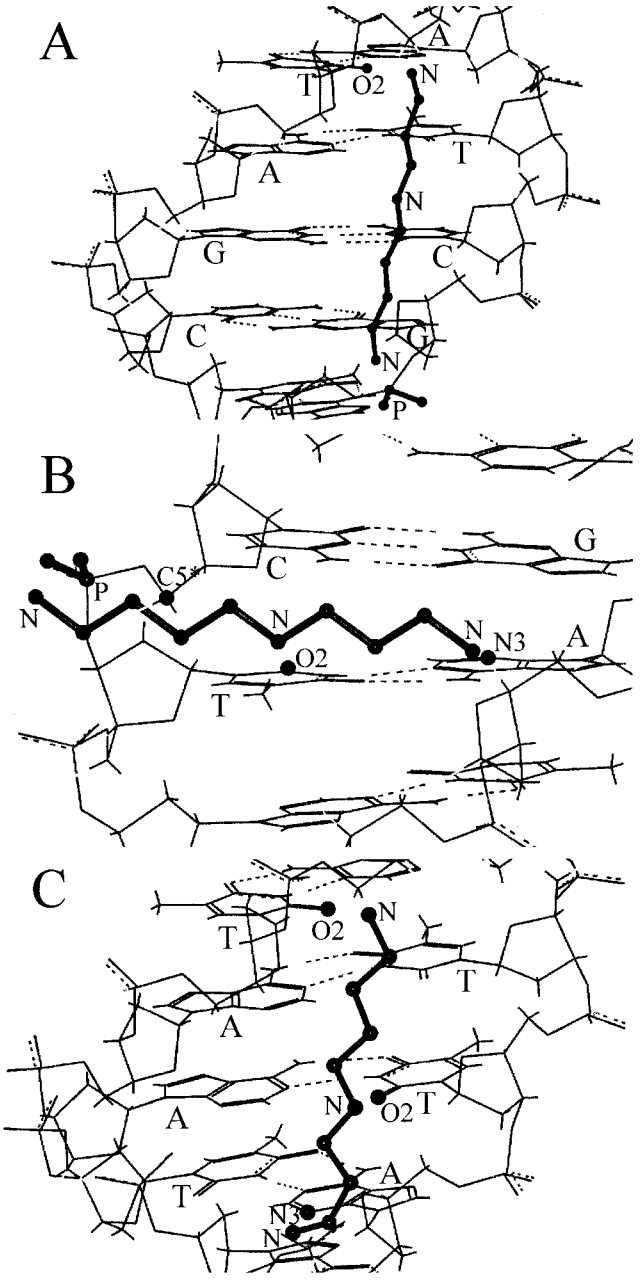

FIGURE 7 Preferential binding model proposed for spermidine-DNA complexation. (*A*) Phosphate and pyrimidine-O2 from different strands of the minor groove; (*B*) Phosphate and purine-N3 from different strands of the minor groove; (*C*) Two pyrimidine-O2 from different strands of the minor groove.

The phosphate-phosphate interstrand distances at the DNA major groove do not favor the attachment of spermidine molecules, supporting the Raman data.

In summary, the binding models proposed for DNA/spermidine complexes together with the Raman results involve both intra- and interstrand interactions. In our opinion, the interstrand interaction would explain the ability of this polyamine to radioprotect DNA and the small hypochromism observed in the difference Raman spectra.

Spermine

As mentioned above, the interaction between spermine and DNA has been widely studied in the past by using different experimental and theoretical techniques (Pattabiraman et al., 1984; Feuerstein et al., 1986, 1990; Drew and Dickerson, 1981; Jain et al., 1989; Williams et al., 1990; Marquet and Houssier, 1988), and several models have been described. These models, however, are often contradictory, and the question concerning the spermine-DNA binding remains troublesome. The observed shifts for the DNA guanine-N7 Raman band at 1488 cm^{-1} upon spermine addition indicate interaction by the major groove, whereas the thymine-O2 band at 1669 cm^{-1} (minor groove) does not appreciably shift. The Raman difference spectrum at spermine concentration 7.5 mM supports this statement. Despite the fact that no shifts have been measured for the phosphodioxy Raman band at 1094 cm^{-1}, the difference spectrum also confirms the existence of NH_3^+-phosphate contacts, as reported by several authors (Drew and Dickerson, 1981; Marquet and Houssier, 1988; Egli et al., 1991; Feuerstein et al., 1986).

In our opinion, the binding model depicted in Fig. 8 *A* presents the following characteristics: 1) it satisfactorily explains both the observed Raman shifts and Raman difference spectra, as a hypochromism effect was not observed for the vibrations of base residues; 2) it is compatible with the estimated distances between the reactive sites of both DNA and spermine; 3) it involves negligible deviations from the all-*trans* polyamine conformation; and 4) the interstrand interaction allows us to explain the DNA radioprotection observed for this polyamine. The polyamine molecule is placed across the major groove with three non-sequence-dependent attachment possibilities: the phosphate moieties for the two outer spermine NH_3^+ groups and a purine-N7 atom for an inner NH_2^+ group. The second NH_2^+ group can interact either with a thymine-O4 or with other purine-N7 atoms, depending on the base sequence; interaction with cytosine residues are, in our opinion, less probable. This conclusion agrees with reported studies about sequence dependence on the spermine-dsDNA interaction, in which significant differences were proposed between A-T and G-C base pairs (Marquet and Houssier, 1988). A second interaction model has been displayed in Fig. 8 *B*, in which the spermine molecule is placed along the major groove attached to a phosphate group and a purine-N7 atom of the same DNA strand. In this model, however, the interactions with the bases are always sequence dependent, and protection from strand breakage would not be as effective as when the interaction involves both strands.

The binding model displayed in Fig. 8 *A* has been observed from crystallographic studies on B-DNA dodecamers stabilized with spermine (Drew and Dickerson, 1981). In addition, it has been theoretically studied using molecular mechanics and DNA oligomers (Feuerstein et al., 1990)

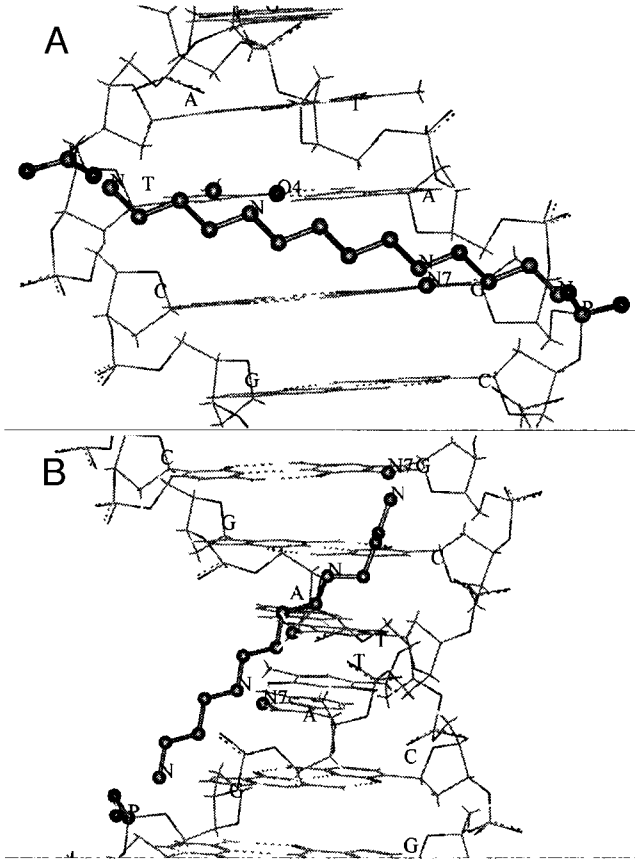

FIGURE 8 Preferential binding model proposed for spermine-DNA complexation. (*A*) Interstrand along the major groove; (*B*) Intrastrand across the major groove.

after a minimization process. These calculations predicted folding of the DNA strands over the polyamine to enhance the interactions, involving broadening of the minor groove and narrowing of the major groove. This hypothesis would explain the monotonical shift upon spermine complexation measured for the phosphodiester Raman band at 838 cm^{-1} observed for solutions in both natural and heavy water. Interaction models in the minor groove were also theoretically studied, being less favorable than the interactions by the major groove (Feuerstein et al., 1990). Our Raman data do not exclude them, although they can be considered as less probable, at least under our experimental conditions. Even exo-groove interactions, being the only binding sites for phosphate groups, are possible as supported by the difference Raman spectra of highly polymerized DNA.

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REFERENCES

Andreasson, B., L. Nordenskiold, and W. H. Braunlin, J. Schultz, and P. Stilbs. 1993. Localized interaction of the polyamine methylspermidine with double-helical DNA as monitored by ^{1}H NMR self-diffusion measurements. *Biochemistry.* 32:961–967.

Basu, H. S., H. C. Schwietert, B. G. Feuerstein, and L. J. Marton. 1990. Effects of variation in the structure of spermine on the association with DNA and the induction of DNA conformational changes. *Biochem. J.* 269:329–334.

Bellamy, L. J. 1981. The Infrared Spectra of Complex Molecules. Chapman and Hall, London.

Benevides, J. M., P. L. Stow, L. L. Ilag, N. L. Incardona, and G. J. Thomas, Jr. 1991. Differences in secondary structure between packaged and unpackaged single-stranded DNA of bacteriophage ϕX174 determined by Raman spectroscopy: a model for ϕX174 DNA packaging. *Biochemistry.* 30:4855–4863.

Benevides, J. M., and G. J. Thomas, Jr. 1985. Dependence of purine 8C-H exchange on nucleic acid conformation and base-pairing geometry: a dynamic probe of DNA and RNA secondary structures. *Biopolymers.* 24:667–682.

Braunlin, W. N., T. J. Strick, and M. T. Record, Jr. 1982. Equilibrium dialysis studies of polyamine binding to DNA. *Biopolymers.* 21: 1301–1314.

Cao, A., J. Liquier, and E. Taillandier. 1995. Infrared and Raman spectroscopy of biomolecules. *In* Infrared and Raman Spectroscopy. B. Schrader, editor. VCH, Wheinheim, Germany. 345–371.

Carmona, P., and M. Molina. 1990. Raman and infrared spectra of D-ribose and D-ribose 5-phosphate *J. Raman Spectrosc.* 21: 395–400.

Cohen., S. S. 1998. Molecular effects on internal cellular polymers: transfer RNA and DNA. *In* A Guide to the Polyamines. Oxford University Press, New York. 512–543.

Douki, T., Y. Bretonniere, and J. Cadet. 2000. Protection against radiation-induced degradation of DNA bases by polyamines. *Radiat Res.* 153: 29–35.

Drew, H. R., and R. E. Dickerson. 1981. Structure of a B-DNA dodecamer. III. Geometry of hydration. *J. Mol. Biol.* 151:535–556.

Egli, M., L. D. Williams, Q. Gao, and A. Rich. 1991. Structure of the pure-spermine form of Z-DNA (magnesium free) at 1-Å resolution. *Biochemistry.* 30:11388–11402.

Erfurth, S. C., and W. L. Peticolas. 1975. Melting and premelting phenomenon in DNA by laser Raman scattering. *Biopolymers.* 14:247–264.

Esposito, D., P. Del Vecchio, and G. Barone. 1997. Interactions with natural polyamines and thermal stability of DNA: a DSC study and a theoretical reconsideration. *J. Am. Chem. Soc.* 119:2606–2613.

Feuerstein, B. G., N. Pattabiraman, and L. J. Marton. 1986. Spermine-DNA interactions: a theoretical study. *Proc. Natl. Acad. Sci. U.S.A.* 83: 5948–5992.

Feuerstein, B. G., N. Pattabiraman, and L. J. Marton. 1990. Molecular mechanics of the interactions of spermine with DNA: DNA bending as a result of ligand binding. *Nucleic Acids Res.* 18:1271–1282.

Ghomi, M., R. Letellier, J. Liquier, and E. Taillandier. 1990. Interpretation of DNA vibrational spectra by normal coordinate analysis. *Int. J. Biochem.* 22:691–699.

Gosule, L. C., and J. A. Schellman. 1976. Compact form of DNA induced by spermidine. *Nature.* 259:333–335.

Ha, H. C., N. S. Sirisoma, P. Kuppusamy, J. L. Zweier, P. M. Woster, and R. A. Casero, Jr. 1998a. The natural polyamine spermine functions directly as a free radical scavenger. *Proc. Natl. Acad. Sci. U.S.A.* 95:11140–11145.

Ha, H. C., J. D. Yager, P. A. Woster, and R. A. Casero, Jr. 1998b. Structural specificity of polyamines and polyamine analogues in the protection of DNA from strand breaks induced by reactive oxygen species. *Biochem. Biophys. Res. Commun.* 244:298–303.

Ha, H. C., P. M. Woster, J. D. Yager, and R. A. Casero, Jr. 1997. The role of polyamine catabolism in polyamine analogue-induced programmed cell death. *Proc. Natl. Acad. Sci. U.S.A.* 94:11557–11562.

Hayashi, S., Y. Murakami, and S. Matsufuji. 1996. Ornithine decarboxylase antizyme: a novel type of regulatory protein. *Trends Biochem. Sci.* 21:27–30.

Heby, O., and L. Persson. 1990. Molecular genetics of polyamine synthesis in eukaryotic cells. *Trends Biochem. Sci.* 15:153–158.

HyperChem, release 5.0, 1996. Hypercube, Ontario, Canada.

Jain, S., G. Zon, and M. Sundaralingam. 1989. Base only binding of spermine in the deep groove of the A-DNA octamer d(GTGTACAC). *Biochemistry.* 28:2360–2364.

Janne, J., L. Alhonen, and P. Leinonen. 1991. Polyamines: from molecular biology to clinical applications. *Ann. Med.* 23:241–259.

Krafft, C., W. Hinrichs, P. Orth, W. Saenger, and H. Welfle. 1998. Raman spectroscopic analysis of tet repressor-operator DNA interaction in deuterium oxide. *Cell. Mol. Biol.* 44:239–250.

Liquori, A. M., L. Constantino, V. Crescenzi, V. Elia, E. Giglio, R. Puliti, M. De Santis-Savino, and V. Vitagliano. 1967. Complexes between DNA and polyamines: a molecular model. *J. Mol. Biol.* 24:113–122.

Lord, R. C., and G. J. Thomas, Jr. 1967. Raman studies of nucleic acids. II. Aqueous purine and pyrimidine mixtures. *Biochim. Biophys. Acta.* 142: 1–11.

Marques, M. P. M., and L. A. E. Batista de Carvalho. 2000. Theoretical approach to the conformational preferences of putrescine. *In* Biologically Active Amines in Food, Vol. 4. D.M.L. Morgan, A. White, F. Sánchez-Jiménez, and S. Bardócz, editors. Office for Official Publications of the European Communities, Luxembourg. 122–129.

Marquet, R., and C. Houssier. 1988. Different binding modes of spermine to A-T and G-C base pairs modulate the bending and stiffening of the DNA double helix. *J. Biomol. Struct. Dyn.* 6:235–246.

McMurry, L. M., and I. D. Algranati. 1986. Effect of polyamines on translation fidelity in vivo *Eur. J. Biochem.* 155: 383–390.

Neault, J. F., M. Naoui, M. Manfait, and H. A. Tajmir-Riahi. 1996. Aspirin-DNA interaction studied by FTIR and laser Raman difference spectroscopy. *FEBS Lett.* 382:26–30.

Overman, S. A., K. L. Aubrey, K. E. Reilly, O. Osman, S. J. Hayes, P. Serwer, and G. J. Thomas, Jr. 1998. Conformation and interactions of the packaged double-stranded DNA genome of bacteriophage T7. *Biospectroscopy.* 4:47–56.

Pattabiraman, N., R. Langridge, and P. A. Kollman. 1984. An iterative approach to placing counterions around DNA. *J. Biomol. Struct. Dyn.* 1:1525–1533.

Pegg, A. E. 1988. Polyamine metabolism and its importance in neoplastic growth and a target for chemotherapy. *Cancer Res.* 48:759–774.

Pelta, J., F. Livolant, and J. L. Sikorav. 1996. DNA aggregation induced by polyamines and cobalthexamine. *J. Biol. Chem.* 271:5656–5662.

Peticolas, W. L. 1975. Applications of Raman spectroscopy to biological macromolecules. *Biochimie.* 57:417–428.

Prescott, B., W. Steinmetz, and G. J. Thomas, Jr. 1984. Characterization of DNA structures by laser Raman spectroscopy. *Biopolymers.* 23: 235–256.

Puppels, G. J., C. Otto, J. Greve, M. Robert-Nicoud, D. J. Arndt-Jovin, and T. M. Jovin. 1994. Raman microspectroscopic study of low-pH-induced changes in DNA structure of polytene chromosomes. *Biochemistry.* 33:3386–3395.

Raspaud, E., M. Olvera de la Cruz, J. L. Sikorav, and F. Livolant. 1998. Precipitation of DNA by polyamines: a polyelectrolyte behavior. *Biophys. J.* 74:381–393.

Rouzina, I., and V. A. Bloomfield. 1998. DNA bending by small, mobile multivalent cations. *Biophys. J.* 74:3152–3164.

Sarhan, S., and N. Seiler. 1989. On the subcellular localization of the polyamines. *Biol. Chem. Hoppe Seyler.* 370:1279–1284.

Spiro, T. J. 1987. Raman spectra on the conformations of biological macromolecules. *In* Biological Application of Raman Spectroscopy, Vol. 1. John Wiley and Sons, New York. 81–133.

Suwalsky, M., W. Traub, U. Shmueli, and J. A. Subirana. 1969. An x-ray study of the interaction of DNA with spermine. *J. Mol. Biol.* 42: 363–373.

Sy, D., Hugot, S., C. Savoye, S. Ruiz, M. Charlier, and M. Spotheim-Maurizot. 1999. Radioprotection of DNA by spermine: a molecular modelling approach. *Int. J. Radiat. Biol.* 75:953–961.

Tabor, H., and C. W. Tabor. 1984. Polyamines. *Annu. Rev. Biochem.* 53:749–790.

Thomas, T. J., and V. A. Bloomfield. 1984. Ionic and structural effects on the thermal helix-coil transition of DNA complexed with natural and synthetic polyamines. *Biopolymers.* 23:1295–1306.

Thomas, T., G. D. Kulkarni, M. A. Gallo, N. Greenfield, J. S. Lew, A. Shirahata, and T. J. Thomas. 1997. Effects of natural and synthetic polyamines on the conformation of an oligodeoxyribonucleotide with the estrogen response element. *Nucleic Acids Res.* 25:2396–2402.

Thomas, G. J., Jr. and M. Tsuboi. 1993. Raman spectroscopy of nucleic acids and their complexes. *Adv. Biophys. Chem.* 3:1–70.

Thomas, G. J., and A. H. Wang. 1988. Laser Raman spectroscopy of nucleic acids. *In* Nucleic Acids and Molecular Biology, Vol. 2. F. Eckstein and D. M. J. Lilley, editors. Springer-Verlag, Berlin. 1–30.

Tsuboi, M. 1964. On the melting temperature of nucleic acid in solution. *Bull. Chem. Soc. Jpn.* 37:1514–1522.

Warters, R. L., G. L. Newton, P. L. Olive, and R. C. Fahey. 1999. Radioprotection of human cell nuclear DNA by polyamines: radiosensitivity of chromatin is influenced by tightly bound spermine. *Radiat. Res.* 151:354–362.

Williams, L. D., C. A. Frederick, G. Ughetto, and A. Rich. 1990. Ternary interactions of spermine with DNA: 4′-epiadriamycin and other DNA: anthracycline complexes. *Nucleic Acids Res.* 18:5533–5541.