On the interpretation of Raman spectra of 1-aminooxy-spermine/DNA complexes

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ABSTRACT

By FT–Raman spectroscopy, we have investigated the effect of 1-aminooxy-spermine (AOSPM) on aggregation and stability of calf-thymus DNA and selected oligonucleotide chains. AOSPM is able to mimic spermine in some macromolecular interactions, but is unable to substitute polyamines to maintain cell proliferation, suggesting pharmacological applications. Raman spectra of solutions containing AOSPM and either genomic DNA or two 15mer oligodeoxynucleotides, with GC or AT sequences, were recorded. Precipitation was observed for calf-thymus DNA, aggregated structures and appearance of several Z marker bands were observed for the 15mer GC sequence, and no macromolecular changes were detected for the 15mer AT sequence. Specific binding sites between the aminooxy group and the base residues were also evidenced. Assignment of the AOSPM Raman bands was supported on a normal mode calculation for the molecule NH₂–O–CH₃ as a model. The theoretical results, in combination with the analysis of the Raman bands, demonstrated that the aminooxy group played a relevant role in the AOSPM–DNA interaction. Preferential binding by the major groove was evidenced in the absence of macromolecular changes. When either precipitation or aggregation occurred, the interaction involved both the major and minor grooves. The specific interaction between AT/GC base pairs and the aminooxy group has also been theoretically investigated. The biological relevance of this work is discussed.

INTRODUCTION

A series of isosteric analogs of the biogenic polyamines has been synthesized recently by replacing the terminal amino-methylene group, NH₂–CH₂–, with the aminooxy one, H₂N–O–(1,2). One of these molecules is the spermine derivative 11-(aminooxy)-4,9-diaza-1-aminoundecane (Supplementary Material), hereafter referred to as AOSPM (aminooxy-

mine). Since the pKa of the aminooxy group is about 5, these polyamine analogs are insufficiently protonated at physiological pH. Consequently, they are suitable molecules for studying the role of structural properties, such as charge distribution or molecular size, in the biological functions of the biogenic polyamines. In fact, it has been shown that the activity of polyamines is largely determined by the location of positively charged nitrogen atoms and the chemical nature of the linkages between these nitrogen atoms (3).

The biogenic polyamines are present in all living organisms (4–6), where they act as metabolic regulators, in cell proliferation and differentiation and as stabilizing agents of nucleic acid structure and conformation (7–9). Polyamines are also known to protect DNA from damage caused by external agents, such as reactive oxygen species or radiation (10,11). Efforts have been made in the past to explain how polyamines bind to nucleic acids. Different DNA–polyamine models have been proposed, from purely electrostatic models (12,13) to those largely based on structural specificities (14,15). In the last few years, Raman spectroscopy has provided us with new data about the nature of this interaction (16,17), which has supported the existence of structure-based binding models.

Aminoxy analogs of biogenic polyamines have been proved as inhibitors or substrates of the enzymes involved in methionine and polyamine metabolism (18,19). They are transported into cells and catabolized by regulated pathways, but have low cytotoxicity and can lack the growth-promoting capability depending on both the analog and the cell type assayed (20–23). It has also been demonstrated that AOSPM is able to provoke DNA aggregation and precipitation (24). These processes are strongly related to DNA folding and dynamics inside the cells. However, it is not yet clear how polyamine analogs interact with DNA and modulate cellular functions and specific gene expression. On the other hand, natural polyamines and polyamine derivatives are under development as DNA delivery vehicles for gene therapy (25). Therefore, due to the important role played by natural polyamines in DNA packaging (26,27) and function (4,28), and the emerging use of polyamine analogs and derivatives as gene delivery vehicles and potential drug candidates for chemotherapy, more information on the structural interactions between DNA and polyamine analogs is necessary.

In recent years, our laboratory has been applying FT–Raman spectroscopy to study the interaction between genomic DNA and biogenic polyamines (17). These experiments have

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demonstrated that polyamine–DNA binding is not exclusively electrostatic, but that chemical interactions are present. Spermine preferentially interacts by the DNA major groove, while spermidine and putrescine show preferential binding to the minor groove. In addition, we also studied the effects of polyamines on 15mer oligodeoxyribonucleotides with alternating AT and GC sequences (29,30). Raman experiments have shown that the biogenic polyamines interact differently depending on the base sequence. Thus, the Ψ (aggregated) and Z (left-handed) forms of DNA were only detected for GC sequences, while interaction by the two DNA grooves was observed for AT sequences. Interestingly, this result can be related to the different roles described for AT- and GC-enriched motifs in the cell cycle processes occurring in vivo, since the AT regions are involved in macromolecular synthesis initiation steps, by allowing the opening of DNA (31); by contrast, the GC sequences are related to compacted structures during gene expression (32).

The aim of the present paper is to obtain structural details of the interaction between DNA and AOSPM using FT–Raman spectroscopy, since understanding of the specific interactions between DNA and AOSPM, and their differences with respect to natural polyamines, could yield valuable information for both basic and applied research. We have used highly polymerized calf-thymus genomic DNA and selected oligodeoxyribonucleotides containing alternating AT and GC sequences. The analysis of changes on the nucleotide Raman bands upon AOSPM addition allowed us to propose preferential binding sites among the interacting systems. The suggested models were also supported by ab initio quantum chemical calculations in two ways. We computed a general quadratic force field for the simplest molecule containing the aminooxy moiety, NH₂-O-CH₃, hereafter referred to as AOM (aminooxy methane), as a model for achieving a reliable assignment of the aminooxy vibrational bands. The solvent–solvent electrostatic interactions were simulated employing a continuum solvent model. On the other hand, the specific interaction between AT/GC base pairs and the aminooxy group was theoretically studied by obtaining the minimal energy structures of the complexes AT–AOM and GC–AOM, which were built in the light of the Raman results. Electronic correlation was introduced in all the calculations from the density functional theory (DFT). Finally, the biological relevance of this work is discussed.

MATERIALS AND METHODS

Materials and Raman measurements

Highly polymerized calf-thymus genomic DNA was purchased from Sigma Chemical Co. UV absorbances at 260 and 280 nm were measured. The obtained A₅₀₀/A₂₈₀ ratio was 1.75, indicating that the DNA had a low protein content. Details about synthesis of AOSPM, as the tetrahydrochloride, are given elsewhere (1,22). The single-stranded 15mer oligonucleotides d[GC(T)₃], and d[AT₃(T)₃], and their anti-parallel and complementary sequences, were synthesized by Pharmacia-Biotec (Sweden). Double-stranded oligonucleotides, d[GC(T)₃]·d[C(G)₃] and d[AT₃(T)₃]·d[T(AT₃)₃], were obtained and tested as previously reported (29). Aliquots of them at 120 mM (in phosphate), using as solvent 20 mM Tris–HCl and 200 mM NaCl, at pH 7.5, were stored at −20°C until recording the spectra.

AOSPM complexes with DNA and the 15mer oligonucleotides, at millimolar concentrations of the aminooxy polyamine, were prepared as described for biogenic polyamines (17,29,30). The selected ranges were justified because millimolar concentrations of polyamines were found in the nucleus of eukaryotic cells (33).

FT–Raman spectra were recorded in a Bruker Equinox 55 Fourier transform spectrometer. Extensive details about recording settings and restrictions to consider and discussion of the Raman peaks can be taken from previous studies (17,29,30). Raman spectra of DNA solutions were normalized between 600 and 1800 cm⁻¹ in order to preserve them from baseline deviations, and were subsequently corrected by subtracting the spectrum of a buffer solution prepared at identical experimental settings. Raman spectra of the oligonucleotide solutions were normalized to the sapphire band at 750 cm⁻¹. No buffer subtraction was performed since the presence of any oligonucleotide changed the optical properties of the solution, as revealed by the fact that some sapphire signals were not fully compensated.

Quantum mechanical calculations and vibrational dynamics

The GAUSSIAN package of programs (34) was used for quantum chemical calculations. We used ab initio methodology at the 6–31G++ level (35,36), which includes polarization functions on all atoms. Electron correlation energy was introduced using the hybrid functional B3PW91 (37,38), which has been successfully proved for biological systems (39–41). To simulate an aqueous environment, a self-consistent reaction field (SCRF) model was employed (42–44). The solvent was assimilated to a continuum characterized by its dielectric constant (78.4 for water), and the solute was placed into a spherical cavity adapted to its size.

Quadratic force constants were computed from the analytical second derivatives of the molecular energy with respect to the cartesian coordinates, in the geometry of minimal energy. The structural optimization process was performed under the same theoretical scheme. To achieve a more useful description of the calculated normal modes, the ab initio force field, in cartesian coordinates, was transformed into a set of non-redundant locally symmetrized internal coordinates, defined following the Pulay methodology (45). Vibrational dynamics were performed using the Wilson FG matrix method (46).

RESULTS AND DISCUSSION

Raman spectra of solutions with calf-thymus genomic DNA

The FT–Raman spectra of DNA, AOSPM and DNA–AOSPM complexes, at identical experimental settings (buffer concentration and pH) are displayed in Figure 1. Relevant wavenumbers and assignments for the complete range of concentrations studied are listed in Table 1. It is known (47) that polycations induce DNA collapse when increasing both the concentration and the molecular charge. Since AOSPM is a polycation at physiological pH (charge > +3), the Raman spectra at the higher AOSPM concentrations of 15 and 30 mM,
were collected from a solid precipitate. This fact gave rise to a strong intensity increase in the oligonucleotide bands because the Raman scattering was collected directly from the solid phases, which usually give rise to a more intense Raman signal than solutions. We would like to emphasize that the AOSPM concentration required to provoke DNA precipitation was similar to spermine (17) under the same experimental settings. However, spermidine (charge = +3) did not induce DNA precipitation for concentrations <50 mM (17). Altogether, these results indicate that the aminooxy group plays an important role in the DNA–AOSPM interaction. Further discussion will allow us to give a structural description to this role. At AOSPM concentrations <10 mM we always obtained homogeneous solutions during the complete recording process.

DNA Raman bands largely arose from base and phosphate vibrations (48–50), which are distributed in three relatively separated regions: from 1700 to 1200 cm⁻¹ (aromatic stretching vibrations of the bases), from 1200 to 800 cm⁻¹ (stretching vibrations of the phosphate moiety) and from 800 to 600 cm⁻¹ (aromatic in-plane bending vibrations of the bases). Deoxyribose moieties give rise to two groups of weak Raman bands, which appear between 1500 and 1400 cm⁻¹ (methylene bending modes) and in the phosphate region (skeletal stretching modes). Proposed assignments in the literature for all of these bands are included in Table 1. In some cases, assignments are described in terms of a specific DNA atom or functional group, which makes the related Raman band very useful for recognition of specific DNA–ligand interactions.

The three bands measured in the Raman spectrum of DNA between 1580 and 1480 cm⁻¹ have been assigned to purine ring stretching vibrations (48,51). The weak peak measured at 1513 cm⁻¹ also contains some cytosine contributions. All of them shifted downwards when adding AOSPM, greater drug concentrations corresponding to greater wavenumber shifts. Nevertheless, at low AOSPM concentrations, that is to say, before DNA collapse, the 1578 cm⁻¹ band scarcely deviated while the band at 1488 cm⁻¹ exhibited its higher shifts with respect to free DNA. This fact could be relevant because the latter has been mainly assigned to the N7 atoms of guanine residues (48,49,51) (located at the DNA major groove). In contrast, the band at 1578 cm⁻¹ has been described with a relevant contribution from the N3 atoms of purine rings (52) (minor groove).

As aforementioned, deoxyribose vibrations often appear as weak Raman bands. An exception is the one measured at 1462 cm⁻¹ for DNA, probably due to contributions from aromatic stretching modes, largely adenine (16). When adding AOSPM this band shifted downwards by 4 cm⁻¹. The strong Raman band at 1376 cm⁻¹ has been reported as a major groove marker peak since it was assigned to methyl bending vibrations of thymine bases (51). Before DNA collapse, it was measured at 1374 cm⁻¹, thus indicating the presence of hydrophobic interactions with the methane groups of AOSPM. However, lower deviations were observed for the solid complexes, as also observed for the 1488 cm⁻¹ guanine N7 band.

The Raman band at 1339 cm⁻¹, which has been assigned to purine stretching vibrations (53), shifted by −2 cm⁻¹ on addition of AOSPM. At the highest concentration used,
Table 2. B3PW91/SCRF=6–31G** vibrational wavenumbers and normal mode descriptions for AOM

<table>
<thead>
<tr>
<th>Vibration</th>
<th>Wavenumber (cm⁻¹)</th>
<th>Potential energy distributionb (values greater than 10%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>v₁</td>
<td>1343</td>
<td>99 τ(NH₂)</td>
</tr>
<tr>
<td>v₂</td>
<td>1266</td>
<td>47 φ(NH₂), 23 ν(CO)</td>
</tr>
<tr>
<td>v₃</td>
<td>1204</td>
<td>66 τ(CH₃)</td>
</tr>
<tr>
<td>v₄</td>
<td>1181</td>
<td>93 τ(CH₃)</td>
</tr>
<tr>
<td>v₅</td>
<td>1031</td>
<td>62 ν(CO), 37 φ(NH₂)</td>
</tr>
<tr>
<td>v₆</td>
<td>911</td>
<td>94 ν(NO)</td>
</tr>
</tbody>
</table>

aArbitrary numbering.
bτ = rocking vibration; φ = umbrella vibration; ν = stretching vibration.

30 mM, it was measured as an intense peak at 1332 cm⁻¹ with a shoulder near 1341 cm⁻¹. Regarding the Raman spectrum of AOSPM (Fig. 1), the 1332 cm⁻¹ band should be better assigned to the 1327 cm⁻¹ one of the aminooxy polyamine. However, a correct interpretation of this deviation when complexing with DNA requires a reliable assignment of this wavenumber.

The lack of vibrational studies about this novel series of compounds encouraged us to perform a normal coordinate calculation for the AOM molecule in a polar environment as a model. First, its molecular structure was optimized until reaching a point of minimal energy. A conformational equilibrium between two main structures, which depended on the relative conformation of the amino group to the methyl one, was obtained. Molecular energy calculations, including solvent energy, demonstrated that the trans conformer is 0.314 kcal/mol more stable than the cis conformer, so it was selected for both the force field calculation and the further discussion of preferential binding sites. Since the calculated potential barrier is low, the structure of AOM in solution will be the result of a conformational equilibrium between these two forms. In Table 2, we have summarized the descriptions for calculated wavenumbers between 900 and 1400 cm⁻¹, in terms of potential energy distributions. Wavenumbers containing the higher contributions for bending vibrations of the amino group were v₁, v₂ and v₅. By correlating with the Raman spectra of AOSPM (Fig. 1A), we conclude that the bands at 1327 and 1065 cm⁻¹ involve relevant contributions from vibrations of the aminooxy moiety, probably coupled with methylene bending modes. Consequently, the aforementioned shift (+5 cm⁻¹) observed for the 1327 cm⁻¹ band of AOSPM when interacting with DNA suggests that the aminooxy group is indeed a reactive site of this biogenic polyamine analog.

The DNA bands at 1304 and 1256 cm⁻¹ exhibited contrary behaviors with respect to both wavenumbers and relative intensities, as can be seen in Table 1 and Figure 1, respectively. The former band showed negligible shifts and a strong intensity decrease, especially at 30 mM AOSPM concentration, while the second shifted upwards and increased its intensity. These two bands were assigned to adenine and cytosine vibrations (53,54). However, the 1256 cm⁻¹ band has been described with important contributions from the exocyclic C6-NH₂ group of the purine rings (55). This is relevant data because this adenine moiety could interact with the oxygen atom of aminooxy groups, thus allowing for interaction between the N7 sites and the aminooxy NH₂ groups.

The Raman spectrum of DNA between 1200 and 800 cm⁻¹ is dominated by vibrations of the phosphate groups (56,57), which are the phosphodiester symmetrical stretching vibration, ν(PCO₂⁻) and the phosphodiester symmetrical stretching vibration, ν(PCO). The associated bands followed a similar trend when adding AOSPM: deviations were only observed in solution, reverting to the original wavenumbers at the higher AOSPM concentrations. As can be observed in Table 1, most of the bands measured between 800 and 600 cm⁻¹, which were assigned to aromatic bending vibrations of the bases (50,58), exhibited significant shifts in the spectra of the precipitated DNA–AOSPM complexes.

Raman spectra of solutions with the 15mer GC oligonucleotide

The Raman spectra of aqueous solutions containing d(GCG)₄-d(CGCG)₄ alone and in the presence of different AOSPM concentrations, from 5 to 75 mM, are shown in Figure 2. Wavenumbers of the more relevant bands and their assignments are summarized in Table 3. In contrast to highly polymerized DNA, no solid precipitation phenomena were observed over the whole range of concentrations studied. This fact is related to the chain length, which is the only factor that

![Figure 2. FT-Raman spectra of solutions in Tris–HCl 20 mM, NaCl 200 mM. (A) d(GCG)₄-d(CGCG)₄ 60 mM (in phosphate); (B) d(GCG)₄-d(CGCG)₄ 60 mM and AOSPM 5 mM; (C) d(GCG)₄-d(CGCG)₄ 60 mM and AOSPM 25 mM; (D) d(GCG)₄-d(CGCG)₄ 60 mM and AOSPM 50 mM; (E) d(GCG)₄-d(CGCG)₄ 60 mM and AOSPM 75 mM.](image-url)
modulates the DNA collapse when the rest of experimental settings are identical. Long nucleotide chains can be precipitated by the effect of a small amount of polyanine molecules suitably located, while short chains would precipitate independently, thus increasing the polyanine/phosphate molar ratio required for collapsing.

The Raman spectrum of poly-ds[dG-dC] was studied by Benevides and Thomas (59), who reported and assigned spectra of both B and Z secondary structures in aqueous solution. The spectrum of our 15mer oligonucleotide (Fig. 2A), fits well with that reported for the polymer in the B conformation, so that the assignments listed in Table 3 were largely taken from poly-ds[dG-dC]. As can be observed in Figure 2, the Raman spectrum of d[G[G(C)]_2]-d[G(C)]_2]-AOSPM, when the spermine analog is used at 75 mM, is rather different to that recorded for the oligonucleotide alone. Two main features can be pointed out.

Firstly, most of the Raman bands exhibited noteworthy intensity decreases (hypochromism); this can be inferred by using an internal standard either the 750 cm⁻¹ band, (sapphire cell), or the broad intense band centered at about 1650 cm⁻¹ (solvent). Thermal denaturation experiments (58) have demonstrated that Raman hypochromism is originated from alterations of the base–base forces, namely the hydrogen bonds (pairing) and the parallel attractions between adjacent π systems (stacking). Polyaniline-induced Raman hypochromism in alternating GC sequences was also observed with the biogenic polyanines (29,30), and was related to the formation of a new tertiary structure named Ψ-DNA (60,61). The proposed model for Ψ-DNA involved an alternating disposal of the oligonucleotide and the polycation chains, thus forming a highly packaged structure (cholesteric), similar to a liquid crystal (61,62). This model has been also named as DNA aggregated. The observed intensity decrease is then justified by the increased pairing and stacking forces in the aggregates.

Secondly, from the comparison between the reported Raman spectra for B and Z conformations of poly-ds[dG-dC] (59) and results shown in Figure 2, it was suspected that AOSPM could induce a conformational change from B-DNA to Z-DNA on the 15mer oligonucleotide used in this work, as a prior step to the formation of Ψ-DNA. The Raman features that can be assigned to this change are: 1420→1424 cm⁻¹ (methylene), 1362→1358 cm⁻¹ (guanine), 1317→1321 cm⁻¹ (cytosine), 828→817 cm⁻¹ (phosphodiester) (48,59,63,64). They can be better observed in Figure 3. Furthermore, it is accepted that the Z form would facilitate Ψ-DNA formation (65–67) since it has two similar grooves, in contrast to the B form. In fact, it has been observed that Z-DNA chains present a strong tendency to self-association (68), being identified as an intermediate in the formation of condensed states of DNA (66).

Raman hypochromism was also observed in the spectrum of the oligonucleotide with 50 mM AOSPM. In addition, several non-conformational marker bands exhibited wavenumber shifts in the presence of AOSPM, as listed in Table 3. These shifts indicate preferential interaction with guanine residues at the major groove. Of particular interest is the behavior of the AOSPM band at 1327 cm⁻¹. It can be correlated to the 1334 cm⁻¹ band measured in the Raman spectrum of the d[G[G(C)]_2]-d[G(C)]_2]-AOSPM (50 mM) solution (Fig. 2D), which appeared as a shoulder of the band at 1321 cm⁻¹ when the AOSPM concentration reached 75 mM. The aforementioned results from ab initio calculations assigned it to a vibration of the aminoxy group, Table 2. Consequently, the observed shifts indicate a direct contribution of the aminoxy moiety in the interaction with the GC oligonucleotide, as inferred for genomic DNA.

Raman spectra of d[G[G(C)]_2]-d[G(C)]_2]-AOSPM solutions also showed a growing band which was measured at 1248 cm⁻¹ at 75 mM of AOSPM concentration. Since the spectrum of the aminoxy polyanine alone did not exhibit any close band, it has to be assigned to guanine or cytosine vibrations. Taking into account that the DNA Raman band at 1256 cm⁻¹ has been assigned to the exocyclic amino groups (51), we have assigned the 1248 cm⁻¹ band to an NH₂ bending vibration involving both guanine (minor groove) and cytosine (major groove). The observed intensity increase was a direct effect of the
interaction between these acidic groups and the AOSPM oxygen atoms.

**Raman spectra of solutions with the 15mer AT oligonucleotide**

The Raman spectra of aqueous solutions containing d[AT]$_3$·d[AT]$_3$ alone and in the presence of AOSPM at concentrations 5, 25 and 75 mM, are shown in Figure 4. Wavenumbers of the more relevant bands and their assignments are summarized in Table 4. The Raman spectrum of this 15mer oligonucleotide (Fig. 4A), exhibited a similar pattern to those reported for poly-ds[dA-dT] (69–73), so we have taken into account the proposed assignments for this polynucleotide. Assignments were also supported by experimental and theoretical studies on the vibrational spectra of adenine (74,75) and thymine (76).

All the spectra displayed in Figure 4 corresponded to double-strands in the B form, and no evidence of macro-molecular changes (precipitation or aggregation) on the AT oligonucleotide was achieved. The Raman spectrum at the highest AOSPM concentration used, 75 mM, only exhibited a slight intensity increase for several oligonucleotide bands. This effect can be originated by a relaxation of the base pairing as a consequence of the interaction. Base-pairing relaxation causes the valence electrons of both base and phosphate moieties to be less attached to the bond regions, thus increasing their ability to polarize and, consequently, the intensities of their Raman bands.

Considering the band measurement, the spectra of d[AT]$_3$·d[AT]$_3$–AOSPM complexes showed relevant wavenumber shifts, with respect to that recorded for the oligonucleotide alone (Table 4). In addition, the AT bands at 1291 and 1187 cm$^{-1}$ showed intensity decrease, while the one measured at 1250 cm$^{-1}$ exhibited greater intensities in the presence of AOSPM. These results indicate that the interaction of AOSPM with d[G-C]$_3$·d[G-C]$_3$ (previous section) seems to be rather different to that with d[AT]$_3$·d[AT]$_3$.

In the light of the assignments listed in Table 4, attachment of AOSPM molecules would be established by the major groove, where they could bind to the N7 and C6-NH$_2$ groups, while preferential interaction by the minor groove was not supported. These results can be analyzed in the light of the structural differences existing between GC and AT sequences. The Raman spectra have evidenced that both the aminooxy moiety of AOSPM and the exocyclic amino groups of the bases are involved in the interaction of this aminooxy polycation with GC and AT sequences. Spectra of DNA–AOSPM solutions also support this assessment. However, the exocyclic amino groups of the bases are present in both the minor and the major grooves of GC double-stranded oligonucleotides, while they are only present in the major groove of AT chains. Interestingly, the proposed models for cholesteric forms of oligonucleotide chains (77–79) suggest that interaction by both oligonucleotide grooves could be a structural requirement for aggregation.

**Analysis of the binding sites**

The results summarized above can be interpreted in terms of specific reactive sites for both the nucleotides and the aminooxy polycation. Taking into account genomic DNA, different interactions were found for different aggregation states. When the AOSPM concentration was lower than ~10 mM, the main spectral changes involved atoms or
functional groups located at the major groove: N7 sites of guanine, exocyclic amino groups of adenine or cytosine and methyl groups of thymine. No spectral evidence was achieved supporting changes on the DNA macromolecular conformation, although relevant wavenumber shifts were observed for the phosphate group vibrations, especially concerning the phosphodiester stretching mode. As described above, AOSPM concentrations $>10$ mM gave rise to DNA collapse into a solid precipitate. Raman spectra obtained from this solid phase showed deviations smaller than those observed in solution for most of the major groove bands. In addition, bands showing small or negligible changes in solution exhibited greater deviations in the precipitate, either in wavenumber or relative intensities: 1578 (N3, purine, minor groove), 1513 (adenine), 1462 (deoxyribose), 1256 (C6-NH$_2$, purine, both grooves) and 753 (thymine) cm$^{-1}$. In the light of these Raman results, preferential binding of AOSPM by the DNA major groove are proposed for the solution complexes, while the DNA collapse seems to need interaction by both DNA grooves. The specific role attributed to the exocyclic amino groups of the bases in the AOSPM-DNA binding can be well correlated with aforementioned shifts for the aminoxy group vibrations. In fact, amino groups of the bases were not putative binding sites for biogenic polyamines, as previously demonstrated (17). However, they can interact with the oxygen atoms of aminoxy moieties, thus allowing for a second contact involving the amino group and a near nucleophilic position of DNA, as N7 (adenine), O6 (guanine) or O2 (cytosine), depending on the specific -O- (AOSPM) -NH$_2$ (DNA) interaction.

The results obtained in the present work for the 15mer oligonucleotides allowed us to support the DNA-based conclusions. At first glance, dramatic differences were found between the effects elicited on GC and AT sequences by the spermine analog. We obtained indications of putative AOSPM-induced conformational transitions on $d(GC)_{15}$-$d(GC)_{15}$, while $d(AT)_{15}$-$d(TA)_{15}$ clearly remained in its canonical B conformation and exhibited changes in a few bands, which involved mainly the major groove. By considering the structural specificities of these two

sequences, interaction by both nucleotide grooves is related again with macromolecular changes: collapse for genomic DNA (highly polymerized) and aggregation for GC oligonucleotides (short chains). When the interaction is located only at one groove, limited changes are observed, which do not involve the macromolecular structure.

Quantum chemical calculations of minimal energy structures

To evaluate the reliability of the binding sites proposed in the preceding section, we have obtained minimal energy structures of two different complexes. One of them was formed by an adenine-thymine base pair and one AOM molecule interacting by the major groove. The second had a guanine-cytosine base pair and two AOM molecules, thus allowing for interaction by both the minor and the major grooves. They were built taking into account the previously discussed Raman results. Structural optimizations were performed in two steps. Firstly, the base pair and the AOM molecule were treated separately, thus obtaining their minimal energy structures without interacting. Secondly, the whole systems, AT-AOM/ GC-AOM, were optimized allowing all the structural parameters to vary independently. Minimal energy structures were finally obtained for the two complexes studied, which maintain the initial network of intermolecular interactions.

The final structure for the AT-AOM complexes is shown in Figure 5. Negligible changes on the Watson-Crick pairing were obtained. As the greatest deviations obtained, the O--H hydrogen bond enlarged by 0.04 Å, while the related O-N angle decreased by 1.7°. Bond lengths and angles for the proposed AT-AOM interactions (Supplementary Material) are within the hydrogen bond ranges, although calculated values for the AOM.O--H.N.A bond indicate a slightly stronger interaction than for the AOM,N.H--N,A one. The interacting atoms preserve the base symmetry plane, the maximal deviation from planarity being 13.4°.

The optimized structure for the GC-(AOM)$_2$ complexes are shown in Figure 6. The main difference with respect to the
AT–AOM complexes involved the two AOM,O–H–N interactions, which are sterically limited in order to adopt a linear conformation, characteristic of the hydrogen bond. The obtained O–H distances, namely 2.08 and 2.19 Å, indicate that interaction is preserved. As can be observed, the main stabilization factors were the two AOM,N–H–O hydrogen bonds, whose angles were 162.9 and 166.6°. A consequence of this result is that the GC base pairing exhibited greater changes with respect to those calculated for the AT pairing (Supplementary Material). The maximal deviation was calculated for the G,O–H–N,C hydrogen bond: the O–H length decreased by 0.08 Å upon AOM complexing, while the bond angle went from 179.2 to 172.2°. Similar changes were obtained for the other two hydrogen bond angles, although the related distances exhibited lower deviations. In this case, the hydrogen bond cooperative effect of the two AOM molecules is a basic requisite, in our opinion, for stabilizing the whole complex.

Preferential binding models

The experimental and theoretical results exposed above can help us to propose preferential interaction models between DNA and AOSPM. To build them, we have selected both AT and GC sequences; relevant intermolecular distances were calculated using standard geometrical parameters (80).

The model proposed for AOSPM–[d(A(TA)₂)·d(T(ATA)₂)] complexes (Supplementary Material) is based on the quantum chemical results, that is, to say, it involves N7 and exocyclic NH₂ sites of adenine at the major groove. The rest of the polyamine chain is adapted to the groove shape. The secondary amino groups of the analog interact with T,C4=O and A,N7 sites, while the primary amino group binds to another C4=O site, in accordance with the Raman results.

In the case of [d(G(CG)₃)·d(C(GC)₃)], the binding of the aminooxy group shown in Figure 6 does not allow the AOSPM molecules an easy adaptation to the oligonucleotide grooves without adopting a highly forced conformation. However, if we accept the extended all-trans conformation, which has been demonstrated as the more stable one for polyamines (81), the primary amino groups of AOSPM molecules could be placed far from the grooves. As a consequence, they could interact with reactive positions of other oligonucleotide chains, as G,N7 atoms, C=O sites or phosphate groups. When the amount of AOSPM was great enough, this model would allow for oligonucleotide aggregation in compact structures, according to the experimental results. In consequence, we propose that the one AOSPM molecule could bind simultaneously to two [d(G(CG)₃)·d(C(GC)₃)] chains; the aminooxy group would interact as depicted in Figure 6, while the amino groups would interact with the reactive sites of both the minor and major oligonucleotide grooves.

Comparison with biogenic polyamines

The results obtained in the present work can be compared to those reported for the biogenic polyamines under the same experimental settings (17). The AOSPM molecule is isosteric to spermine, and has the same positive charge, at physiological pH, as spermidine. Our previous Raman studies (17) on the interaction of these two biogenic polyamines with DNA showed noticeable similarities between spermine and AOSPM. Thus, both polyamines induced DNA collapse at concentrations higher than ~10 mM, and spectra also indicated preferential interactions by the DNA major groove in solution. By contrast, DNA–spermidine solutions were studied up to spermidine concentrations of 50 mM without observing solid phases, and the Raman spectra led us to deduce a preferential interaction by the minor groove. Thus, our results support that the DNA–polyamine interaction has an important structural dependence and, more specifically, binding with the bases was evidenced.

The role of molecular structure is enhanced when comparing the results obtained with both spermine and AOSPM solutions in the presence of short oligonucleotide chains. As mentioned above, the AT minor groove is not suitable for interaction with the aminooxy group because the main reactive sites are electron donors. Since the Raman spectra of AT with both spermine or spermidine indicated preferential interaction by the minor groove (30), this different behavior has to be related to the presence of the aminooxy group, which is the only structural change introduced. Whether this structural difference is related to the lack of function of the analog as a growth promoting polyamine (23) is a tentative hypothesis needing further studies.

Finally, we focus our attention on the obtained results for the 15mer [d(G(CG)₃)·d(C(GC)₃)]. This oligonucleotide collapsed when the spermine concentration was higher than ~25 mM, while no solid phases were observed with either spermidine or AOSPM at a concentration of 75 mM (29). At these concentrations, the corresponding Raman spectra exhibited a similar hypochromism, which evidenced the presence of highly-ordered structures (Y-DNA). However, the Raman features related to B→Z transition were weaker for both biogenic polyamines than for AOSPM. Thus, the roles of both the molecular charge and the structural specificities are evidenced. It has been demonstrated that a B-DNA sequence can host more spermine units per base pair than the same nucleotide as Z-DNA (82). Our results provide experimental evidence that the presence of the aminooxy group extended the binding possibilities of AOSPM molecules with the base residues with respect to the biogenic polyamines.

Biological significance

The essential role of polyamines for maintaining cell growth has been widely documented over the last 20 years (83). The nuclear concentration of DNA is very high and of the order of 200–400 mg/ml (84). The cellular DNA is in a macromolecular crowded environment, surrounded by proteins and cationic molecules, including polyamines. As demonstrated recently (85), an important percentage of spermine is located in the cell nucleus. Since polyamines associate with DNA through preferential binding modes (17), a possible function of polyamines in the cell might involve the organization of the genetic material. One of the early discoveries about polyamine–DNA interactions was the observation that polyamines could stabilize double-stranded DNA (86). Other effects related to polyamine–DNA interactions relevant to cellular functions in vivo include induction of B→Z transitions, DNA bending and condensation (28,47,79,87–90). Furthermore, polyamines and derivatives can be used as condensing agents to package genes of interest for efficient transfection into living cells (28). These facts have encouraged different research groups to study differences and similarities
in the interactions of DNA with different natural polyamines and analogs.

Evidence existed pointing to AOSPM as a very promising polyamine analog (23,24). It is demonstrated that AOSPM enters mammalian cells by competing with polyamines for the same uptake systems, so helping to deplete cells of the natural compounds. In addition, DNA binding properties could be suspected since the compound was able to protect DNA against oxidative damage, as also occurs with spermine (23). This compound shows unique structural features to characterize the role of the charged aminopropyl group of spermine in DNA binding. Thus, by comparing the preferential binding modes of spermine with those of AOSPM, new insight can be provided on the spermine structure--(growth promoting) function relationships. For instance, the aminopropyl group allows spermine to bind to the minor groove of an AT-enriched sequence, but this is not favored in the case of AOSPM. The ability of spermine to bind to both grooves in AT-enriched sequences must favor denaturation of these sequences during nucleic acid initiation processes, and it could be related to its growth promoting ability, in contrast to AOSPM. The observed differences between spermine and AOSPM to modify the DNA macromolecular structure could also provide interesting information for a deeper characterization of the molecular bases of the growth-promoting capacity of spermine.

The results shown in this work can also shed light on biomedical applications. The results altogether, particularly those indicating aggregation of DNA, and therefore showing the capability of AOSPM to form cholestatic DNA structures, along with its inability to promote cell growth, reinforce the previous hypothesis of its potential application in the development of improved non-viral vector delivery strategies for gene therapy against cancer and other proliferative pathologies (23,28,79).

SUPPLEMENTARY MATERIAL
Supplementary Material is available at NAR Online.

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