Raman spectroscopy study of the interaction between biogenic polyamines and an alternating AT oligodeoxyribonucleotide

J. Ruiz-Chica a, M.A. Medina b, F. Sánchez-Jiménez b, F.J. Ramírez a, *

a Departamento de Química Física, Facultad de Ciencias, Universidad de Málaga, Málaga 29071, Spain
b Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga, Málaga 29071, Spain

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Abstract

The interaction between the 15-mer oligonucleotide d[AT]7 d[TA]7 and the three biogenic polyamines, putrescine, spermidine and spermine, under physiological conditions has been studied by Raman spectroscopy. Solutions containing 60 mM (phosphate) of the oligonucleotide and different polyamine concentrations ranging from 1 to 75 mM have been studied. Both natural and heavy water were used as solvents. Difference Raman spectra were computed by subtracting the sum of the separated component spectra from the experimental spectra of the complexes. The Raman data suggested that the interaction of biogenic polyamines with d[AT]7 d[TA]7 presents differences related with their sizes and electric charges. Preferential bindings through the oligonucleotide minor groove for putrescine and spermidine were proposed. Spermine would interact by both minor and major grooves, although interaction by the minor groove seems to be more favored. Main reactive sites were thymine-O2 and adenine-N3 atoms at the minor grooves and adenine-N7 and thymine-O4 at the major groove. Electrostatic attractions between the polyamine amino and oligonucleotide phosphodioxy groups were also proposed. Under our experimental conditions, no macromolecular effects on d[AT]7 d[TA]7 (changes on secondary or tertiary structures) were detected from Raman spectroscopy, contrary to what happened for GC sequences at the same experimental settings. This fact agrees with the role of the biogenic polyamines during the first steps of the macromolecular synthesis, which involve DNA opening in AT motifs.

Keywords: Raman; Polyamine; AT; Oligonucleotide; Interaction

1. Introduction

Biogenic polyamines spermine, spermidine and putrescine are low molecular weight polycations present in all living cells [1]. They play important roles in cell growth and differentiation since they can modulate gene expression [1–4]. Other functions of polyamines have been related to DNA protection from external agents [5,6], DNA packaging [7], etc. The negative charge of the DNA backbone makes it a target for cationic species, so direct interaction between biogenic polyamines and DNA was suggested in order to explain some of their biological properties [4]. It has been also proved that interaction of polyamines with DNA gives rise to condensation and/or changes in its secondary structure [8,9]. However, the nature of the polyamine–DNA binding at a molecular detail is not clearly established, being currently the subject of great controversy. Thus, it has been proposed from Raman studies on spermine and spermidine with genomic DNA that the polyamine–DNA interaction is largely electrostatic, base sequence playing only secondary roles [10]. Recent molecular dynamics simulations have nevertheless indicated that spermine strongly interacts with different DNA moieties [11,12]. It is also known that X-ray diffraction techniques have not undoubtedly detected spermine–DNA associations in crystals, with the exception of the early work of Drew and Dickerson [13]. Nevertheless, evidence achieved up to now indicates that a purely electrostatic model cannot successfully explain polyamine–DNA interaction.

For the last years, our laboratory has paid attention to this topic. Reported Raman data for genomic DNA allowed us to suggest preferential binding models for the three biogenic polyamines in which they interacted with the DNA bases [14]. The proposed models involved spermine–DNA asso-
ciations by the major groove, the positively charged groups of spermine interacting with purine,N(7) and thymine,O(4) reactive sites of DNA. Spermidine and putrescine were preferentially located at the DNA minor groove, with close contacts with pyrimidine-O(2) and purine,N(3) sites. Interaction with the negative phosphodioxy groups was also present. Those results also suggested that the base sequence could play nonvanished roles in polyamine/DNA interactions. Feuerstein et al. [15] evaluated spermine interactions with different alternating AT and GC sequences by using molecular mechanical calculations. They concluded that interaction with the bases was more favorable than interaction along the phosphate backbone in all the oligonucleotides studied. In addition, they achieved differences between GC and AT sequences when interacting with spermine. This fact was also studied by Schmid and Behr [16], using photoaffinity cleavage. They obtained that different polyamines, including polyamines, have similar sequence selectivities. Consequently, they concluded that the interaction of polyamines with DNA is largely guided by the electronegative potential along the nucleic acid, which favors the minor groove of AT-rich regions. The alternating purine–pyrimidine sequence is commonly found in genomic DNA, playing a relevant role in DNA conformational equilibrium, and consequently in replication and gene expression regulation [17,18]. In this way, it is worthwhile to emphasize the structural differences between GC and AT sequences and some of their consequences. The minor groove in AT regions is narrower and more electronegative than in GC regions [19]. Moreover, the absence of the G,N(2)H2 group, Fig. 1, prevents from steric hindrance, so it will be a more reactive site for electrophilic reagents, as polycations, in an AT sequence than in a GC one. On the other hand, the A,N(6)H2 groups provoke steric hindrance on A,N(7) sites at the major groove of an AT oligonucleotide. The importance of this fact comes since these are the only reactive positions free from inter-strand hydrogen bonds at the major groove. Consequently, GC sequences will favor major groove interactions with electrophilic reagents with respect to AT sequences. At the light of these facts, it is not surprising that biogenic polyamines interact differently with AT- and GC-rich regions, as previously proposed [15,20]. In addition, it is also relevant in the cell cycle processes occurring in vivo, since the different roles described for AT- and GC-enriched motifs. Thus, while the AT sequences are involved in the macromolecular synthesis initiation steps, by allowing the opening of DNA [21], the GC sequences are related to DNA compaction during gene expression [22].

In a recent paper we have published results of a Raman spectroscopy study on the interaction between biogenic polyamines and an alternating double-stranded GC oligodeoxyribonucleotide [23]. The spectra indicated the formation of aggregates phases in solution, named as Ψ-DNA. These DNA aggregates are characterized by a high tertiary structure order, which has been related with the DNA packaging in chromosomes [24–26]. Although the main stabilizing factor of DNA aggregates is the electrostatic attraction [27–29], the influence of relevant structural specificities has been also suggested [30,31]. In agreement with this hypothesis, our results on GC strands evidenced interactions with the bases [23]. The purpose of this work is to extend our knowledge about the effect of the polyamines on genomic DNA by studying the interaction of biogenic polyamines with a self-complementary DNA 15-mer containing the repetitive AT sequence. The paper has been organized as follows. First, the Raman spectra of the AT oligonucleotide in H2O and D2O solutions will be analysed and assigned. Second, we will describe the main features of the Raman and difference Raman spectra of solutions containing the AT oligonucleotide and biogenic polyamines. Finally, these features will allow us to analyse the preferential reactive sites and describe some binding models.

2. Materials and methods

2.1. Chemicals and oligonucleotides

The single-stranded 15-mer d[A(TA)7] and its antiparallel and complementary sequence, d[T(AT)7], were synthesized by Pharmacia-Biotech (Sweden). Double-stranded formation was carried out by heating at 95 °C for 10 min in 20 mM Tris–HCl and 200 mM NaCl, at pH 7.5, followed by a slow cooling until reaching the room temperature. This procedure ensures the formation of double strands, as

![Fig. 1. The Watson–Crick pairing in alternating AT oligodeoxyribonucleotide.](image-url)
demonstrated previously for a $^{32}$P labelling GC 15-mer [23]. Aliquots of $d[A(TA)_{7}]\cdot d[T(AT)_{7}]$ 120 mM (phosphate) were stored at $-20$ °C until recording the spectra. Spermine, spermidine and putrescine as polyhydrochlorides were purchased from Sigma Chemical. Polyamine solutions were prepared using the same solvent as for the oligonucleotide. Polyamine–oligonucleotide complexes were prepared by mixing 1.5 µl of each polyamine solution with 1.5 µl of the oligonucleotide solution, thus obtaining 3 µl of solutions 60 mM (phosphate) of $d[A(TA)_{7}]\cdot d[T(AT)_{7}]$ at different polyamine concentrations. Solutions at the same oligonucleotide concentration using Tris–D$_2$O buffer were also prepared.

2.2. Raman spectroscopy

Fourier transform (FT) Raman spectra were recorded at room temperature in a Bruker Equinox 55 Fourier transform spectrometer supplied by a germanium diode detector cooled with liquid nitrogen. Spectra were obtained at a resolution better than 2 cm$^{-1}$. Excitation radiation wave number was 1064 nm, as generated from a Nd-YAG laser working at 500 mW. Three microlitres of each solution was introduced in a 0.8-mm-diameter capillary. The capillary was then placed into a spherical cuvette of 10-mm diameter, made of sapphire, with reflecting internal surface. Thus, the laser beam passes several times through the sample within the cuvette since multiple reflections, making possible to record good quality spectra from very-low-volume liquids. A minimum of 2000 scans was accumulated in all the cases to enhance the signal-to-noise ratios. No local heating was detected, as deduced by comparing accumulations from different times. Individual scans were examined by the recording routine, being automatically discarded when the mean intensity deviations were greater than 12.5% over the full interferogram length. This procedure prevents from artifacts in the resulting spectra prior averaging, and was performed at least with two independently prepared samples for each concentration. To discuss the Raman peaks, the following criteria were adopted: (i) peaks must appear with the same wave number and intensity in spectra of independent samples; (ii) peaks must exhibit an intensity clearly greater than the experimental noise level; and (iii) peaks should be previously reported and assigned in the literature. Spectral treatment was performed using the Bruker OPUS C spectroscopic software. All the Raman spectra were normalized to the intense sapphire band at 644 cm$^{-1}$.

Difference spectra were computed by subtracting the sum of separately measured $d[A(TA)_{7}]\cdot d[T(AT)_{7}]$ and polyamine spectra from the corresponding experimental spectra of the complexes, each of them previously normalized and corrected from baseline deviations. The following criteria were employed to discuss the Raman difference features: (i) the difference bands should exhibit an intensity ratio at least twice the noise level; (ii) the difference features must be structurally interpretable; and (iii) the difference features must be reproducible for independently prepared samples. In addition, both H$_2$O and D$_2$O solutions should support conclusions.

3. Results and discussion

3.1. Raman spectra and assignments of $d[A(TA)_{7}]\cdot d[T(AT)_{7}]$

The Raman spectra of $d[A(TA)_{7}]\cdot d[T(AT)_{7}]$ solutions (60 mM in phosphate, 20 mM Tris–HCl buffer, 200 mM NaCl) in H$_2$O and D$_2$O are shown in Fig. 2. They are fully compatible with those reported for poly-ds(dA–dT) in the B-form, as reported by different authors [32–36]. Consequently, we assume this secondary structure for our 15-mer.
oligonucleotide. Table 1 summarizes the observed wave numbers and proposed assignments. They were largely based on the proposed assignments for the polymer, in addition to some reported experimental and theoretical studies on the vibrational spectra of the isolated base residues, adenine [37,38] and thymine [39–41].

Bands appearing between 1700 and 1200 cm⁻¹ were assigned to stretching vibrations of the base residues, with the exception of those measured between 1470 and 1400 cm⁻¹, which correspond to methylene and methyl bending vibrations. The intense Raman band at 1667 cm⁻¹ from D₂O solution was assigned to a C=O stretching vibration from thymine residues. Both the C2=O and the C4=O bonds are expected to be involved in the associate normal mode [36], in addition to some stretching vibrations from the pyrimidine ring. This assignment is also supported by reported normal coordinate analysis for thyme in D₂O solution [39] on the basis of ab initio calculations. Major contributions from the C2=O bonds to the intensity of the 1667 cm⁻¹ band can be assumed since these groups are not involved in the Watson–Crick hydrogen bond pairing (see Fig. 1). Their outer electrons will be therefore less attached to the valence regions than those directly involved in hydrogen bonds, thus increasing polarizability and, consequently, Raman intensities.

No wave numbers were reported from the vibrational spectra of thymine between 1490 and 1670 cm⁻¹ [39–41].

<table>
<thead>
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<th>D₂O Wave numbera</th>
<th>Assignment</th>
</tr>
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<tbody>
<tr>
<td>1607 w 1605 m</td>
<td>1577 m 1576 s</td>
<td>adenine (purine stretching)</td>
</tr>
<tr>
<td>1510 s 1512 s</td>
<td>1478 vv 1483 vv</td>
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<td>1420 w 1422 m</td>
<td>deoxyribose skeletal</td>
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<td>1375 m 1376 m</td>
<td>1341 m 1343 m</td>
<td>adenine (purine stretching)</td>
</tr>
<tr>
<td>1302 m 1303 s</td>
<td>1291 w 1292 w</td>
<td>adenine, thymine</td>
</tr>
<tr>
<td>1250 w</td>
<td>1187 w</td>
<td>adenine, thymine</td>
</tr>
<tr>
<td>1092 vs 1092 vs</td>
<td>928 vv 929 vv</td>
<td>phosphodiroyx, PO₂⁻, stretching</td>
</tr>
<tr>
<td>793 m 793 m</td>
<td>729 m 725 m</td>
<td>adenine (purine in-plane bending, N3)</td>
</tr>
</tbody>
</table>

*Raman bands at 1607, 1577 and 1510 cm⁻¹ from H₂O solutions were therefore assigned to purine ring stretching vibrations, in agreement with previous assignments proposed for poly-ds(dA–dT) [32,36] and genomic DNA [42,43]. They were reported at 1612, 1597 [38] and 1505 cm⁻¹ [44,45] for solid adenine, being all of them assigned as stretching vibrations of the purine ring. Theoretical calculations of the adenine vibrational spectrum [38] demonstrated that the normal mode for the band at 1607 contained important contributions from N(7), N(9) and N(3) atoms. The one associated to the 1577 cm⁻¹ band had significant contributions from both the N(6)H₂ bending vibration and an aromatic stretching mode with elevated amplitude for the N(3) atom. Since the NH₂ group is not a putative site of interaction for cations, observed changes on this band when adding polyanines will be better attributed to interactions with the N(3) atoms of adenine, which are located at the oligonucleotide minor groove.

The Raman band at 1478 cm⁻¹ could contain contributions from both pyrimidine and purine ring stretching vibrations, at the light of theoretical calculations [38,40,41]. It appeared at 1483 cm⁻¹ in the solution in D₂O, Table 1, as was also reported for poly-ds(dA–dT) [36]. Since the wave number associated to a specific vibrational coordinate must not shift upwards upon deuteration, this fact has to be interpreted as a change in the involved normal coordinate respect to the H₂O solution, which is not surprising for a complex vibrational mode like this. Other Raman bands useful for DNA recognition were measured at 1375 and 729 cm⁻¹. The former was undoubtedly assigned to a methyl bending vibration from thymine residues; this group is located at the oligonucleotide major groove, Fig. 1. The band at 729 cm⁻¹ has been previously assigned to a purine ring vibration [32,33,35]. No Raman bands were reported for thymine in H₂O solution between 833 and 419 cm⁻¹ [39], although the Raman spectra of solid thymine showed lines near at 760 and 740 cm⁻¹ [39–41]. However, they were reported as weak bands, while the Raman spectrum of adenine showed a very strong band at 722 cm⁻¹ [37,38,43,46], which was described having major contributions from C(4)–N(3) bonds [38]. In addition, this band has been reported as the second more intense band of the Raman spectrum of poly(dA) [46]. Finally, the intense bands measured at 1092 and 793 cm⁻¹ were assigned respectively to PO₂⁻ and O−P−O symmetric stretching modes [14,43,47,48]. Wave number sets from deuterated and nondeuterated samples were correlated to a single set of assignments, Table 1, applying a main vibrational contribution approach.

3.2. Raman spectra of d[A(TA)₇]–d[T(AT)₇]–polyamine complexes in H₂O

In order to analyse the influence of the biogenic polyamines on the oligonucleotide d[A(TA)₇]–d[T(AT)₇], the Raman spectrum of Fig. 2A was compared with those obtained for polyamine–oligonucleotide solutions in normal
They were prepared under the same experimental settings as described in the previous section. As aforementioned, different polyamine concentrations were used. We selected the 10–75-mM range for putrescine and spermidine, and the 1–25-mM range for spermine, in order to avoid precipitation of the oligonucleotide [27]. Similar ranges were also studies for genomic DNA [14] and d[G(CG)7]-d[C(GC)7] [23], which will also allow us to establish comparisons. The selected ranges are physiologically justified since millimolar concentrations of polyamines have been found to be associated to nucleic acids in vivo [49,50]. Since the greater deviations were observed for the higher polyamine concentrations in each case, we have displayed in Figs. 3 and 4 the Raman spectra and the Raman difference spectra, respectively, for d[A(TA)7]-d[T(AT)7] in the presence of 75 mM of putrescine and spermidine and 25 mM of spermine. Relevant wave numbers for the complete set of concentrations studied are summarized in Table 2.

In the region where stretching vibrations from the base residues appear, 1700–1200 cm\(^{-1}\), main spermine-induced wave number shifts are related to the bands at 1577 (A,N(3)), 1420 (CH\(_2\) bending) and 1291 (A,T) cm\(^{-1}\). As a general rule, greater spermine concentrations gave rise to greater wave number shifts. Thus, the oligonucleotide bands at 1607 (A), 1375 (T,CH\(_3\)) and 729 (A,N(3)) cm\(^{-1}\) did not appreciably shift at low spermine concentrations, while they went to 1610, 1376 and 728 cm\(^{-1}\), respectively, at 25 mM spermine. The significance of the thymine–methyl band comes since it is the only major groove Raman maker band with intensity enough to ensure a suitable wave number measurement. The second classical major groove maker band, 1478 cm\(^{-1}\) (purine,N(7)), only exhibits a high Raman intensity when guanine residues are present. For d[A(TA)7]-d[T(AT)7], it could also have some contributions from pyrimidine moieties, appearing as a weak band which was often masked by the polyamine methylene bending bands. Concerning phosphate vibrations, the phosphodioxy (PO\(_{2}\)) stretching band at 1092 cm\(^{-1}\) was measured at 1091 cm\(^{-1}\).

![Fig. 3. Raman spectra in normal water of: (A) d[A(TA)7]-d[T(AT)7] at the same conditions as in Fig. 2; (B) the oligonucleotide in the presence of 75 mM of putrescine; (C) the oligonucleotide in the presence of 75 mM of spermidine; (D) the oligonucleotide in the presence of 25 mM of spermine.](image)

![Fig. 4. Difference Raman spectra and relevant wave numbers (in cm\(^{-1}\)) obtained from solutions in normal water: (A) putrescine 75 mM; (B) spermidine 75 mM; (C) spermine 25 mM.](image)

**Table 2**

| Wave numbers (in cm\(^{-1}\)) for the more relevant bands measured in the Raman spectra of d[A(TA)7]-d[T(AT)7]—polyamines in solution of natural water |
|---|---|---|---|---|
| Putrescine (mM concentration) | Spermidine (mM concentration) | Spermine (mM concentration) |
| AT, 60 mM (phosphate) | 10 | 25 | 75 | 10 | 25 | 75 | 1 | 10 | 25 |
| 1607 | 1607 | 1607 | 1610 |
| 1577 | 1576 | 1577 | 1576 | 1577 | 1576 | 1576 | 1577 | 1576 |
| 1510 | 1510 | 1511 | 1509 | 1509 | 1508 |
| 1420 | 1421 | 1422 | 1423 |
| 1375 | 1375 | 1375 | 1376 |
| 1341 | 1340 | 1339 | 1339 | 1341 | 1341 | 1340 |
| 1291 | 1290 | 1290 | 1288 |
| 1250 | 1251 | 1250 | 1252 | 1250 | 1251 | 1252 |
| 1187 | 1186 | 1187 | 1184 |
| 1092 | 1092 | 1091 | 1091 |
| 729 | 729 | 729 | 728 | 729 | 729 | 728 | 729 | 728 | 728 |
cm\(^{-1}\) when adding spermine, while no appreciable wave number shifts were measured for the phosphodiester (O–P–O) stretching band at 793 cm\(^{-1}\).

The measured features in the Raman difference spectrum of this oligonucleotide in the presence of spermine 25 mM are compatible with the aforementioned wave number shifts. Positive bands at 1606, 1573 and 726 cm\(^{-1}\) confirmed interaction with A,N(3) sites, which are the main reactive positions at the oligonucleotide minor groove, while the positive band at 1091 (+) cm\(^{-1}\) was assigned to the PO\(_2\) stretching vibration. Hydrophobic interaction with thymine methyl moieties is supported by features at 1376 (+) and 1360 (–) cm\(^{-1}\), while the band at 1503 (+) cm\(^{-1}\) could be assigned to the purine,N(7) vibration with some thymine contributions. Both T-CH\(_3\) and A,N(7) are located at the oligonucleotide major groove.

Main wave number shifts upon spermidine addition involved to the oligonucleotide bands at 1577 (A,N(3)), 1510 (A), 1250 (A,T), 1187 (d,A,T) and 729 cm\(^{-1}\) (A,N(3)). Contrary to that observed for spermine, the bands assigned to PO\(_2\) stretching and thymine methyl bending modes did not exhibit measurable deviations. In the Raman difference spectrum (75 mM spermidine), the broad band around 1100 cm\(^{-1}\) was due to a baseline difference between experimental and theoretical spectra, and consequently it has not been interpreted. Features at 1605 (+), 1573 (+), 1564 (–) and 728 (–) cm\(^{-1}\) supported interaction with A,N(3) sites by the minor groove. The bands at 795 (–) and 836 (+) were assigned to phosphodiester backbone vibrations, while no appreciable features were observed for the T-CH\(_3\) vibration. The bands at 1482 (–) and 1470 (+) have been assigned to methylene bending vibration of deoxyribose moieties.

Raman spectra of d[A(TA)\(_7\)]·d[T(AT)\(_7\)]–putrescine solutions showed small wave number shifts for the oligonucleotide bands at 1577 (A,N(3)), 1510 (A), 1341 (A) and 729 cm\(^{-1}\) (A,N3). This fact would indicate a weaker interaction of putrescine with this oligonucleotide than spermine or spermidine. The difference Raman spectrum at a putrescine concentration 75 mM exhibited similar features to those for spermidine. Interaction with adenine is mainly supported by the bands at 1603 (+), 1246 (+), 1232 (–) and 730 (–) cm\(^{-1}\). The features at 1246 and 1232 cm\(^{-1}\) were also assigned to thymine vibrations, while those at 795 (–) and 834 (+) cm\(^{-1}\) correlated well with those measured for the d[A(TA)\(_7\)]·d[T(AT)\(_7\)]–spermidine solutions.

The Raman spectra at the higher concentrations studied in each case allowed us to measure several bands between 1500 and 1400 cm\(^{-1}\) that were assigned to the methylene bending vibrations of biogenic polyamines, namely 1477 and 1457 cm\(^{-1}\) for spermine, 1456 cm\(^{-1}\) for spermidine and 1455 cm\(^{-1}\) for putrescine. They deviate by 2–5 cm\(^{-1}\) with respect to the reported wave numbers from the Raman spectra of the free polyamines [14], thus supporting the existence of interaction with the oligonucleotide.

3.3 Raman spectra of d[A(TA)\(_7\)]·d[T(AT)\(_7\)]–polyamine complexes in D\(_2\)O

Raman spectra of solutions in D\(_2\)O, prepared under the same conditions as in the previous sections, allowed us to support the results in H\(_2\)O and extend our analysis to the 1600–1700 cm\(^{-1}\) region. As for the solutions in H\(_2\)O, we have displayed in Figs. 5 and 6 the Raman spectra and Raman difference spectra of the solutions containing the oligonucleotide and the higher polyamine concentrations in each case, while Table 3 summarizes the relevant wave numbers for discussing. The C=O stretching vibrations of an AT sequence only involve to thymine residues (see Fig. 1). The Raman spectrum of d[A(TA)\(_7\)]·d[T(AT)\(_7\)] in D\(_2\)O showed an intense band in this region measured at 1667 cm\(^{-1}\), which was largely assigned to the T,C=O stretching mode, as aforementioned. Upon spermine complexation, this band shifted to low wave numbers, greater deviations corresponding to greater concentrations. A similar trend was observed for spermidine while smaller shifts were measured when adding putrescine, respectively. Preferent interaction with C2=O groups at the oligonucleotide minor groove would successfully correlate with the most significant spectral features described for solutions in H\(_2\)O.

Other spectral changes measured for spermine–d[A(TA)\(_7\)]·d[T(AT)\(_7\)] solutions in D\(_2\)O involved the bands at 1576, 1512, 1343 and 725 cm\(^{-1}\). They were all largely

![Fig. 5. Raman spectra in heavy water of: (A) d[A(TA)\(_7\)]·d[T(AT)\(_7\)] at the same conditions as in Fig. 2; (B) the oligonucleotide in the presence of 75 mM of putrescine; (C) the oligonucleotide in the presence of 75 mM of spermidine; (D) the oligonucleotide in the presence of 25 mM of spermine.](image-url)
assigned to adenine stretching and bending vibrations; those at 1576 and 725 cm\(^{-1}\) are also considered as minor groove maker bands. The Raman band at 1512 cm\(^{-1}\) is accompanied by a shoulder measured at 1503 cm\(^{-1}\), which was also assigned to a purine stretching vibration. Upon spermine addition, both of them shifted to higher wave numbers. Thus, they were measured at 1512 and 1503 cm\(^{-1}\), respectively, at a spermine concentration of 1 mM. When increasing the spermine concentration, some overlapping was observed, although the bands always shifted upwards. The Raman band at 1154 (d,A,T) cm\(^{-1}\) shifted to higher wave numbers, being measured at 1159 cm\(^{-1}\) at a spermine concentration of 25 mM. The intense PO\(_2\) peak, whose wave number was not affected by the hydrogen–deuterium exchange, exhibited the same trend for H\(_2\)O and D\(_2\)O solutions, while the T,CH\(_3\) band at 1376 cm\(^{-1}\) shifted upwards by 1–2 cm\(^{-1}\) upon spermine complexation. Difference Raman spectra for spermine–d[A(TA)\(_7\)]\(\rightarrow\)d[T(AT)\(_7\)] show a negative band at 1664 cm\(^{-1}\), which indicates an intensity change of the T,C(2) = O band, thus supporting interaction by this reactive site. Features at 1605 (–), 1578 (+), 1344 (–), 1319 (+) and 722 (–) cm\(^{-1}\) are related to adenine vibrations, while the positive band at 1360 cm\(^{-1}\) has been assigned to the T,CH\(_3\) vibration.

The Raman spectra of spermidine–d[A(TA)\(_7\)]\(\rightarrow\)d[T(AT)\(_7\)] solutions in D\(_2\)O showed significant wave number shifts for the oligonucleotide bands at 1576, 1512, 1422 and 725 cm\(^{-1}\), which can be well correlated with measured deviations in H\(_2\)O. Difference features at 1674 (+), 1665 (–), 1642 (+) and 1630 (–) cm\(^{-1}\) supported interaction with C=O groups of thymine residues. Interaction with adenine was indicated by the bands at 1573 (+), 1348 (–), 1329 (+) and 721 (–) cm\(^{-1}\). Features at 820 (+) and 812 (–) cm\(^{-1}\) have been assigned to phosphodiester backbone vibrations.

Concerning putrescine, wave number shifts largely involved the bands at 1576 (A), 1512 (A), 1422 (d), 1292 (A,T) and 929 (d) cm\(^{-1}\). As observed for spermidine, no shifts were measured for the phosphodi oxy stretching and thymine methyl bending vibrations. The difference spectrum at a putrescine concentration 75 mM exhibited features at 1664 (–) and 1638 (+) cm\(^{-1}\), which can be assigned to carbonyl stretching vibrations from thymine residues. Bands at 1573 (–) and 720 (–) cm\(^{-1}\) involved A,N3 sites, while those at 792 (–) and 777 (+) cm\(^{-1}\) were assigned to phosphodiester stretching vibrations. Interaction with adenine was also supported by the negative band at 1347 cm\(^{-1}\), which can be well correlated with the spermidine difference features.

### 3.4. Polyamines–d[A(TA)\(_7\)]\(\rightarrow\)d[T(AT)\(_7\)] interaction

The results described above can be discussed in the light of the proposed assignments for the Raman bands; by considering both spectral changes and difference features, and interaction of the three biogenic polyamines with the bases. In addition, the magnitude of the shifts can lead us to specific interactional sites for each polyamine. Thus, preferential binding of spermidine and putrescine by the oligonucleotide minor groove can be proposed. As aforementioned, alternating AT-rich regions favor interaction at the minor groove for proton donors, which agrees with this conclusion. However, our results seem to indicate that spermine and spermidine were more effective than putrescine in binding to AT sequences. This fact is in

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**Table 3**

<table>
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<tr>
<th>AT, 60 mM (mM concentration)</th>
<th>Putrescine (mM concentration)</th>
<th>Spermidine (mM concentration)</th>
<th>Spermine (mM concentration)</th>
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![Fig. 6. Difference Raman spectra and relevant wave numbers (in cm\(^{-1}\)) obtained from solutions in heavy water: (A) putrescine 75 mM; (B) spermidine 75 mM; (C) spermine 25 mM.](image-url)
agreement with the reported higher capacity and major role of the former polyamines to sustain macromolecular synthesis and cell growth [51]. It is also supported by reported data in the literature about association constants of polyamines with different nucleotide systems. DeStefano et al. [52] have obtained quantitative data of polyamine–nucleotide complexes in aqueous solution. As an example, the formation percentages for adenosine monophosphate at an ionic strength \( I = 0.15 \) mol\( \cdot l^{-1} \) were 4.6, 12.1 and 15.4 for putrescine, spermidine and spermine, respectively. In all the cases studied (other nucleotides, lower ionic strength and different pH values), the order putrescine < spermidine < spermine was maintained for both formation percentages and formation constants. Similar results were obtained for DNA and shorter oligonucleotides [53,54].

By considering the spectral features for bands which can be related to specific reactive sites, polyamine amino groups would preferentially bind to \( T \cdot C \) and \( A \cdot N3 \) reactive positions. No spectral evidence of interaction by the major groove was achieved for spermidine and putrescine. For spermine, the spectral data suggested binding by both major and minor groove. Evidence of minor groove binding is relevant since previous Raman studies on genomic DNA [14] and \([G\{CG\}_2]-[C\{GC\}_2]\) [23] indicated preferential binding by the DNA major groove. We would like to point out that interaction of spermine with the bases at the major groove has been demonstrated by X-ray studies performed on spermine–oligonucleotide complexes [13,55–57]. Hydrophobic contacts between the methylene groups of spermine and the methyl groups of thymine were also observed [55]. It is known that the more reactive position at the DNA major groove is the purine,\( N(7) \) atom; however, this site is not sterically favored when the purine residue is adenine because of the presence of the \( N(6)H_2 \) amino groups; Fig. 1. In addition, the absence of guanine residues prevents the minor groove from steric hindrance, which also leads to preferential interaction by this groove. Theoretical results reported by Feuerstein et al. [15] established that the stabilization energy for the interaction of spermine with the minor groove in AT sequences was up to \( 50 \) kcal/mol higher than in GC sequences. The whole set of reported Raman data (DNA, GC and AT sequences) seems to indicate, therefore, that the spermine–DNA binding at the major groove is largely induced by the GC-rich regions, in agreement with recent theoretical results [12].

By comparing present results with those obtained for \([G\{CG\}_2]-[C\{GC\}_2]\) [23], the main difference comes from the macromolecular effects induced by polyamines (changes on secondary or tertiary structures). Under our experimental settings, these effects were not detected on \([A\{TA\}_7]-[T\{AT\}_7]\), while \([G\{CG\}_2]-[C\{GC\}_2]\) underwent to an aggregated state in the presence of spermine and spermidine. This conclusion correlates well with reported studies on the binding of spermine to AT and GC base pairs by electronic spectroscopy [20], which suggested that spermine can induce bending in the AT-rich regions, while GC sequences underwent stiffening in the presence of this polyamine. The inability of the biogenic polyamines to cause macromolecular aggregation of an AT oligonucleotide fits well with the biological role of these motifs, which are present in the open complexes occurring in vivo during macromolecular synthesis initiation steps [21]. In fact, S phase of the cell cycle required the presence of polyamines [58]. Theoretically, aggregations of AT-enriched motifs induced by polyamines should retard cell cycle progression or even have lethal consequences, and obviously it is not the case [59]. On the contrary, GC-enriched sequences are typically described as regulators of gene expression through changes in compaction of their containing structures [22], and polyamines indeed have the ability to stabilize aggregated states [23,60]. It is also noteworthy that reported polyamine concentrations in cell nuclei [50] are in the order of those described to induce aggregation on GC oligonucleotides [23] but not on AT oligonucleotides (present work) under the same experimental conditions.

Raman data also indicated that the \([A\{TA\}_7]-[T\{AT\}_7]-\)polyamines interaction involved deoxyribose moieties. This is, in our opinion, an indirect result of the insertion of polyamine molecules between the oligonucleotide strands, better than the consequence of direct links. Difference features for phosphodiester vibrations support this conclusion. In relation to the negatively charged phosphodioxy groups, the characteristic PO2\_ stretching vibration only showed small shifts upon polyamine addition. Although some reported studies have indicated DNA-phosphates as primary binding sites for polyamines [10], these links have been described as quite dynamic [61], which would explain the small deviations observed for the phosphodioxy band in both H2O and D2O. Previous data reported for genomic DNA [14] and a GC oligonucleotide [23] agree with the present work.

### 3.5. Preferential binding models

In this section we will use the structural details described above on the \([A\{TA\}_7]-[T\{AT\}_7]\)–polyamines linkages to propose models of interaction. We referred to them as preferential models under our experimental settings, thus indicating that they do not exclude the existence of other putative models. To reach them, we have also taken into account some relevant intermolecular distances. The lowest energy conformations for these polyamines are the all-\( trans \) structures, as theoretically predicted from \( ab \) initio calculations [61]. The distances between the outer primary amino groups in the all-\( trans \) conformation are 6.2, 11.2 and 16.2 Å for putrescine, spermidine and spermine, respectively. The distance between a primary amino group
and a secondary amino group separated by a trimethylene chain is therefore around 5 Å. The oligonucleotide structure was obtained, using the HyperChem set of programs [62], from standard geometrical parameters for a double-stranded deoxyribonucleotide in the B-type secondary structure, which was the relevant conformation of our 15-mer A–T oligomer as evidenced by the Raman spectra.

In the binding models proposed for putrescine–d[AT( TA)7]–d[T( TA)7] complexes, the primary amino groups of the polyamine could interact with a PO2– group and either T,C(2) = O or A,N(3) sites of the same strand at the minor groove, which are separated by about 5.6 and 5.7 Å, respectively. A putative inter-strand model would involve exclusively to reactive sites of the base residues. Exo-groove interaction by two consecutive phosphodioxymoi-
eties (6.5 Å) cannot be nevertheless discarded.

In the light of the Raman data, the spermidine–d[AT( TA)7]–d[T( TA)7] interactions would involve similar reactive sites than for putrescine. However, the presence of a secondary amino group extends the binding possibility of spermi-
dine. A model could involve binding of the primary amino groups with PO2– and A,N(3) sites of different strands (10.8 Å), which allows for interaction between the inner secondary amino group and the T,C(2) = O sites. A second binding possibility would place the polyamine along the oligonucleotide axes, involving two A,N(3) and a T,C(2) = O reactive positions. Relevant interatomic lengths for this model are 6.4 Å, N(3)–O(2), and 4.3 Å, O(2)–N'(3), which correlate well with those of spermidine, namely 6.2 Å, N(1)H3+–N(5)H5+ and 5 Å, N(5)H2–N(8)H3+.

Spectral data for spermine–d[AT( TA)7]–d[T( TA)7] solutions involved oligonucleotide reactive sites placed at both the minor and the major grooves. In a major groove model, the outer primary amino groups could bind with PO2– and T,O(4) sites from different strands, thus allowing for inner interactions with A,N(7), T,O(4) and T,CH3 sites. A second model would place the spermine molecule along the oligonucleotide axes, in a similar form than spermidine. In this case, two inner interactions between NH2 and T,C(2) = O groups are also involved. We would like to point out that this model requires that the polyamine conformation is slightly bent, instead of the all-trans model used for the other cases. This possibility would be favored by the greater conformational flexibility of spermine with respect to shorter polyamines. Different spermine structures when interacting with DNA have been obtained from X-ray experiments [13,55,56,63] and theoretical studies [11,15], which went from the all-trans conformation to strongly bent ones. They support the idea that, in solution, the spermine molecules use their conformational flexibility to bind with oligonucleotide chains. However, the reported structures showed a high dependence on factors such as the oligonucleotide sequence, the solvation settings or the specific location of spermine on the double-helix, so that correlations with the interaction in solution could be risked.

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