Interaction of DNA with an aminooxy analogue of spermidine — an FT-IR and FT-Raman approach

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Abstract

The interaction of highly polymerized calf-thymus DNA with 1-aminooxy-3-\textit{N}-(3-aminopropyl)-aminopropane (ap-apa), an aminooxy analogue of the biogenic ornithine-derived polyamine spermidine, has been investigated by vibrational spectroscopy. Infrared and Raman spectra of DNA/ap-apa solutions, at different polyamine concentrations, were registered. The infrared spectra were extended to solutions in heavy water in order to analyze the 1500–1700 cm\textsuperscript{-1} region. The spectroscopical data were discussed in terms of preferential binding sites between DNA and the aminooxy polyamine. The results support the existence of structural specificities in the interaction, at least under our experimental conditions; they also indicate differences at low and high ap-apa concentrations, which involve mainly base residues. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

Biogenic ornithine-derived polyamines are essential molecules for all living organisms, from microorganisms to superior plants and animals [1,2]. They have different physiological roles, mainly as metabolic regulators [3]. To explain some of these functions, direct interaction between polyamines and DNA has been suggested [4]. Although the exact nature of the polyamine–DNA interaction remains unknown up to now, the experimental evidence achieved in the last years leads to alternative binding sites for a specific polyamine–DNA system, which could be correlated with the polyamine functions.

Among the different ways to improve our knowledge about the biological roles of natural polyamines, the use of structural analogues has demonstrated to be a powerful tool. In order to study the roles of charge distribution and structural requirements in the molecular physiology of natural polyamines, a series of compounds in which the carbon atom next to the amino nitrogen is substituted by oxygen have been synthesized [5,6]. In this paper, we present a study by FT-Raman and FT-IR spectroscopy of the interaction between DNA and 1-aminooxy-3-\textit{N}-(3-aminopropyl)-aminopropane, ap-apa, in solution. In a previous paper, the interaction between DNA and spermidine had been investigated by vibrational
spectroscopy [7], giving preferential binding sites, which can be correlated with those proposed for this aminooxy analogue.

As can be seen in Fig. 1, ap-apa is obtained by replacing one of the outer positively charged ammonium groups of the biogenic polyamine spermidine by a non-protonated (at physiological pH) aminooxy group. This approach for modification of spermidine backbone produces an isosteric and charge insufficient analogue that is able to penetrate into cells, competing for transportation with putrescine. Ap-apa has been shown to be a substrate of SSAT and an inhibitor of spermidine synthase [8]. It is also able to inhibit the growth of BHK, L1210 and Caco-2 cells [9,10]. However, the interactions of this compound with nucleic acids have never been studied prior to this work.

2. Experimental

Highly polymerized calf thymus DNA sodium salt (6.2% sodium content; 13.0% H2O content) was purchased from Sigma Chemical Co. To check the protein content of DNA, ultraviolet absorbances at 260 and 280 nm were measured. The A260/A280 ratio was 1.75, indicating a low protein content. Details about synthesis of the analogue 1-aminooxy-3-N-(3-aminopropyl)-aminopropane trihydrochloride are given elsewhere [5,6].

Solutions were prepared at different ap-apa concentrations. To preserve physiological pH and 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 with hydrogen chloride. Ap-apa/DNA complexes, with polyamine concentrations ranging from 1 to 50 mM, were prepared by adding DNA to the ap-apa solutions, up to 2% final DNA concentration (0.06 M, phosphate). Millimolar concentrations of polyamines have been found in the nucleus of eukaryotic cells [11], thus justifying the selected concentration range. The mixtures were stirred slowly 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 (two days maximum). Solutions were prepared using H2O and D2O (99% D, Aldrich) as solvents.

Vibrational spectra were recorded in a Bruker Equinox 55 Fourier Transform spectrometer supplied with a Raman module. Infrared spectra of the solutions were registered using a demountable cell for liquid samples with calcium fluoride windows, and purging the spectrometer with dry nitrogen gas. A minimum of 500 scans with 2 cm⁻¹ spectral resolution were accumulated in all the cases. Raman spectra were obtained by using excitation radiation wavelength at 1064 nm from a Nd-YAG laser working at 500 mw. Approximately 1 ml solution was placed in a quartz cell for liquids. Backscattering collection of the Raman radiation was performed using a mirror behind the cell, and a minimum of 2000 scans was accumulated in all the cases. The best spectral resolution was 1 cm⁻¹.

Spectral treatment was performed by using the Bruker OPUS spectroscopic software. Infrared spectra were corrected from strong solvent absorptions by subtracting the spectrum of water. Raman spectra were normalized between 600 and 1800 cm⁻¹, to preserve them from baseline differences, and were subsequently corrected by subtracting the spectrum of a buffered solution prepared at identical experimental settings. No further spectral treatment was performed after subtracting.

3. Results

Raman spectra of DNA/ap-apa complexes, at different ap-apa concentrations between 1 and
50 mM, are shown in Fig. 2. They also include spectra of both calf-thymus DNA and polyamines under the same conditions (buffer concentration and pH) as the complexes. The pK\text{a} of an aminooxy group is about 5, so that ap-apa is a bivalent cation in our experimental conditions and, consequently, no precipitation phenomena have been observed in any case, as described in Ref. [12].

Vibrational spectra of DNA have been extensively studied up to now, and assignments for most of the infrared and Raman bands have been reported [13–16]. Upon polyamine complexation, some of those bands show intensity changes and/or wavenumber shifts, which can be interpreted in terms of preferential binding sites between the polyamine and DNA. With regard to Raman spectra, Fig. 2, the measured shifts lead to some differences in the DNA/ap-apa interaction, which depend on ap-apa concentration. Fig. 3 shows the observed trends in the whole range studied for the more significant Raman bands. At ap-apa concentration lower than about 5 mM, the greatest wavenumber shifts are observed for the DNA bands at 1669 (T-O2), 1513 (A), 1304 (A, T) and 753 cm\textsuperscript{-1} (T). However, at ap-apa concentrations higher than 5 mM, the most significant shifts involve

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Fig. 2. Raman spectra of ap-apa, calf-thymus DNA and DNA/ap-apa complexes at different ap-apa concentrations from 1 to 50 mM.
the bands at 1578 (−2, G), 1488 (−2, G-N7), 1462 (−3, G), 1179 (−4, C, T) and 683 cm\(^{-1}\) (+4, G).

No shifts have been measured for both the phosphodioxy, \(\text{PO}_2^\text{2}\), and phosphodiester, O–P–O, symmetric stretching vibrations that appear as intense Raman peaks at 1094 and 788 cm\(^{-1}\), respectively. A second O–P–O stretching DNA band, which appears at 838 cm\(^{-1}\), does shift when ap-apa is added. This is a weak band composed of several components that cannot be split at standard spectroscopical resolution [16]. However, being considered as a DNA conformational marker band, it is very sensitive to conformational transitions. Upon polyamine complexation, it shifts downward up to 2 cm\(^{-1}\). In addition, a new peak at 814 cm\(^{-1}\) can be clearly measured in the Raman spectra at the highest ap-apa concentrations studied, namely 30 and 50 mM.

The DNA/ap-apa interaction has been also studied by infrared spectroscopy, in order to confirm the Raman results. Strong infrared absorptions of the solvent molecules make it difficult to obtain data in the region around 1600 cm\(^{-1}\) from solutions in natural water. This problem can be avoided by recording infrared spectra in heavy water. Both natural and heavy water solutions enable us for a complete infrared study of nucleic acids. In Fig. 4 we show the results for DNA/ap-apa solutions at 5 and 30 mM polyamine concentrations, as examples, along with the infrared spectrum of DNA. The antisymmetric \(\text{PO}_2^\text{2}\) stretching vibration is Raman inactive; however,
it can be observed as a strong band of the DNA infrared spectrum at 1225 cm\(^{-1}\). The measured wave-numbers upon polyamine complexation deviate by 1–2 cm\(^{-1}\). This is not surprising, as the antisymmetric \(\text{PO}_2^-\) stretching mode is more sensitive to environmental and conformational changes than the corresponding symmetrical vibration. As an example, it shifts by 35 cm\(^{-1}\) in A–B–Z transitions [13], while the symmetrical Raman active mode at 1094 cm\(^{-1}\) only shifts by 7 cm\(^{-1}\) [16]. Concerning the interaction with the bases, the infrared spectra support the Raman results. Thus, the DNA bands at 1297 (A), 1282 (T) and 1051 cm\(^{-1}\) (T) shift downwards by 2–3 cm\(^{-1}\) when the polyamine concentration is lower than 5 mM, showing smaller deviations at higher concentrations, as can be seen in Fig. 4.

In the region between 1700 and 1500 cm\(^{-1}\), absorptions at 1522 (G,C), 1536 (C) and 1618 cm\(^{-1}\) (C), undergo greater shifts at 30 mM ap-apa concentration, while the DNA band at 1644 cm\(^{-1}\) shows a significant intensity increase. This band has been assigned to a C=O stretching vibration, largely from G and C residues, supporting the Raman results. Finally, the strong DNA band at 1569 cm\(^{-1}\) (A) presents a shoulder at lower wavenumbers; it increases at 5 mM ap-apa concentration measured at 1563 cm\(^{-1}\).

4. Discussion

The aforementioned infrared and Raman results can be discussed in terms of preferential sites of binding between DNA and ap-apa in solution at physiological conditions, and can be subsequently correlated with reported results for the related biogenic polyamine spermidine. Spectroscopical data evidence interactions with bases, although they establish some concentration-dependent differences. At low ap-apa
concentrations, the greater deviations are related with adenine and thymine residues. More specifically, the wavenumber shifts observed for the thymine-O2 band indicate interaction by the DNA minor groove. In addition, no intensity or wavenumber changes are observed at low ap-apa concentrations for the guanine-N7 and thymine-CH\textsubscript{3} bands, which are considered as significant indicators of interaction by the DNA major groove. This is in agreement with previous results obtained for spermidine [7]. The DNA/ap-apa interaction would therefore occur by binding the outer NH\textsubscript{1} group with the adenine-N3 atom, while the aminooxy moiety could interact with an external PO\textsubscript{2} group of a different strand. This model allows for secondary contacts between the inner NH\textsubscript{2} group of the polyamine and another thymine-O2 atom. Hydrophobic interactions with the methylene-C5' group can also occur, although weakly as deduced from the observed shift (−1 cm\textsuperscript{−1}) for the 1447 cm\textsuperscript{−1} Raman band at low ap-apa concentration.

At high ap-apa concentrations, the observed shifts for the Raman bands at 1488, 1462 and 683 cm\textsuperscript{−1} confirm that the DNA/ap-apa interaction is extended to the major groove, largely involving the guanine residues. Interaction with the DNA major groove has not been proposed for spermidine as a preferential binding model. However, infrared spectra of both \textit{H}\textsubscript{2}O and \textit{D}\textsubscript{2}O solutions support this statement. As aforementioned, one of the most reactive sites at the DNA major groove is the guanine-N7 atom. Raman and infrared results indicate that the ap-apa molecules could also bind by other reactive sites, as purine-O6 or cytosine-N4. In addition, the presence of an oxygen atom in ap-apa allows it to interact with the NH\textsubscript{1} groups of adenine and cytosine, which also lies at the major groove. A binding model that would agree with these data would involve interactions of ap-apa NH\textsubscript{3}\textsuperscript{+} and NH\textsubscript{2}\textsuperscript{+} groups with the guanine-O6 and purine-N7 atoms, respectively, while the phospho-dioxy groups could bind to the ap-apa aminooxy moieties.

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