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Study by electronic circular dichroism spectroscopy of the interaction between aminooxy analogues of biogenic polyamines and selected oligonucleotides

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Abstract

The interaction between a series of aminooxy analogues of the biogenic polyamines spermine and spermidine and selected 15-mer oligodeoxyribonucleotides with alternating purine–pyrimidine base sequences, adenine–thymine (AT) and guanine–cytosine (GC), has been studied using electronic circular dichroism (CD) spectroscopy. These analogues resulted from the substitution of the two terminal aminomethylene groups of the polyamines, $–CH_2NH_2^+$, by an aminooxy one, $–ONH_2$. Since spermidine has no centre of symmetry, it gives rise to two different isosteric molecules, which have been named AOEPUT and APAPA. On the contrary, spermine gives rise to a single aminooxy analogue, named as AOSPM. As the pK_a of an aminooxy group (about 5) is not high enough to be protonated at a neutral pH, these analogues have a positive charge less than the corresponding polyamine under physiological settings, which makes them suitable models to investigate the roles of the charge and the structure in the polyamine–DNA interaction. At low pH values, both the biogenic polyamine and their aminooxy analogues have a similar positive charge.

The CD spectra of solutions containing different concentrations of the three aminooxy analogues and a 15-mer oligonucleotide, containing either the GC or the AT sequence, at a fixed concentration 60 μM in phosphate, were recorded. Solutions at pH values 7.5 and 5.0 were studied in order to investigate the role of the molecular charge. The spectra demonstrated that the interaction of these oligonucleotides with the aminooxy analogues had a significant sequence-selectivity. Spectra of the oligonucleotides in the presence of AOSMP showed two isodicroic points, thus indicating the presence of different oligonucleotide conformations in solution. The CD spectra of AOEPUT and APAPA supported the non-equivalent role that the outer ammonium groups of spermidine, N1 and N8 positions, could have in the interaction of this biogenic polyamine with DNA.

Keywords: GC; AT; Aminooxy; Polyamine; CD

1. Introduction

The essential role of ornithine-derived polyamines for maintaining cell growth has been widely documented over the last 20 years [1]. As demonstrated recently [2], an important percentage of spermine is located in the cell nucleus, so a possible function of polyamines could involve the organization of the genetic material by means of direct interactions with DNA [3–6]. Among the different techniques developped up to now in order to study this interaction, the synthesis of structural analogues to biogenic polyamines has proved to be a very useful tool. Substitution of a terminal aminomethylene group, $NH_2–CH_2–$, by the aminooxy one, $NH_2–O–$, gives rise to a series of isosteric and charge deficient (at physiological pH) analogues, which have been proposed as suitable models for investigating the structural specificities of polyamines required for their physiological functions [7,8]. They have been proposed as useful agents for stabilization of gene therapy vectors, so that these molecules have potential biotechnological applications. Some of them have proved to be inhibitors

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or substrates of the enzymes involved in methionine and polyamine metabolism [7,9]. On the other hand, previous studies have indicated that DNA–polyamine interactions exhibit oligonucleotide sequence dependence [10–12]. This fact has been related to the different roles described for different DNA motifs in cell cycle processes. Thus, adenine–thymine (A–T) regions are involved in DNA opening occurring during the macromolecular synthesis initiation steps [13], while guanine–cytosine (G–C) regions have been related to the regulation of gene expression [14].

In previous works, we have demonstrated that biogenic polyamines interact differently with short G–C and A–T sequences [15,16]. The aim of this paper is to investigate, using electronic circular dichroism (CD) spectroscopy, the interaction of these oligonucleotides with aminooxy analogues of the biogenic polyamines spermine and spermidine. In contrast to spermine, the molecule of spermidine has no centre of symmetry. In consequence, alternating substitution of the two terminal aminomethylene moieties by the aminooxy group gives rise to two different analogues. Fig. 1 shows the chemical structures of the three aminooxy analogues studied, which have been named as AOSPM, 11-[(amino)oxy]-4,9-diaza-1-aminoundecane, AOEPUT, 7-[(amino)oxy]-5-aza-1-aminoheptane, and APAPA, 7-[(amino)oxy]-4-aza-1-aminoheptane. CD spectra of solutions containing the 15-mer oligonucleotides, either $d[G(CG)_7]\cdot d[C(GC)_7]$ or $d[A(TA)_7]\cdot d[T(AT)_7]$, and the aminooxy analogues were recorded. Different oligonucleotide/drug molar ratios were studied at both physiological and acidic pH. The observed features on the oligonucleotide CD bands, upon addition of these molecules, will be structurally interpreted in terms of changes in the oligonucleotide macromolecular conformation.

2. Experimental

The single-stranded 15-mer oligonucleotides $d[G(CG)_7]$ and $d[A(TA)_7]$, and their antiparallel complementary sequences, $d[C(GC)_7]$ and $d[T(AT)_7]$, were synthesized by Pharmacia-Biotech (Sweden). The double-stranded oligonucleotides $d[G(CG)_7]\cdot d[C(GC)_7]$ and $d[A(TA)_7]\cdot d[T(AT)_7]$ were obtained and tested as previously reported [15,16]. Details about synthesis of the aminooxy analogues of biogenic polyamines are given elsewhere [17,18]. Solutions at two different aminooxy concentrations, namely 250 and 1000 μM, were prepared for both $d[G(CG)_7]\cdot d[C(GC)_7]$ and $d[A(TA)_7]\cdot d[T(AT)_7]$ in a 10 mM TRIS buffer, 10 mM sodium chloride. The oligonucleotide concentration was always 60 μM (in phosphate). For each aminooxy concentration, pH values of 7.5 and 5.0 were adjusted using hydrogen chloride.

Samples were kept at 4 °C, and the CD spectra were recorded within 48 h after preparing the solutions. The CD spectra were recorded at room temperature (22 °C) in an JASCO J-810 circular dichroism spectrometer. Standard quartz cells of 1 cm path-length were used for all measurements. Spectra were recorded after buffer

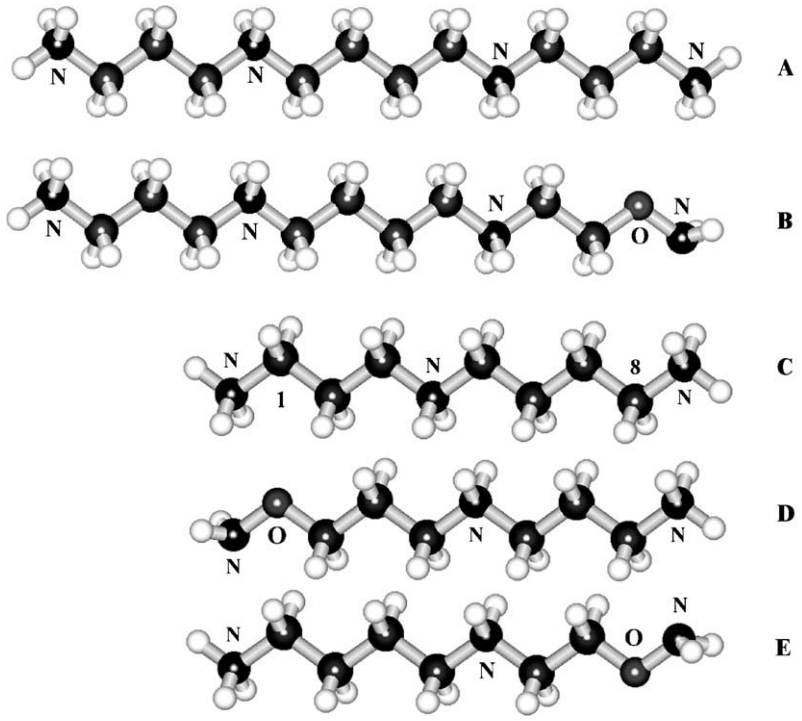

Fig. 1. Chemical structures of spermine (A), spermidine (C) and the three aminooxy analogues studied in this work AOSPM (B), APAPA (D) and AOEPUT (E).

correction. CD spectra corresponded exclusively to the oligonucleotides, since none of the aminooxy analogues had measurable CD signals. The molar ellipticity, $[\Theta]$, was calculated from the equation: $[\Theta]=\Theta/cl$, where Θ is the ellipticity, c is the DNA molar concentration and l is the cell path-length in cm [19].

3. Results and discussion

3.1. Effect of pH lowering on the CD spectra of the oligonucleotides

Figs. 2 and 3 show the CD spectra of the oligonucleotides studied at pH values from 7.5 to 1.5. At physiological pH, the spectra exhibited standard features of a B-DNA conformation [20,21]. When decreasing pH, the most outstanding changes observed for $d[G(CG)_7]\cdot d[C(GC)_7]$ were: the band at 285 nm shifted by +3 nm and exhibited a weak intensity increase; the negative features at 253 and 206 nm showed significant ellipticity decreases, shifting upwards by 3 and 2 nm, respectively. These changes are largely originated by the partial protonation of the citosine-N3 positions ($pK_a=4.24$) [22], which are involved in the Watson–Crick hydrogen bonding, as can be observed in Fig. 4. Further pH lowering provoked these phenomena to be enhanced, and the CD signal almost disappeared at pH values lower than 3.0.

The CD spectrum of the oligonucleotide $d[A(TA)_7]\cdot d[T(AT)_7]$ at pH 5.0 only showed a small hypochromism with respect to the spectrum at physiological pH. Greater changes were observed at pH 3.0, and the signal disappeared at pH 1.5. These results indicate that, at pH 5.0, both oligonucleotides preserve the secondary structure they have at physiological pH; partial protonation can be expected in some basic positions, which gives rise to a slight destabilization of the double strands, especially in the case of the G–C chains.

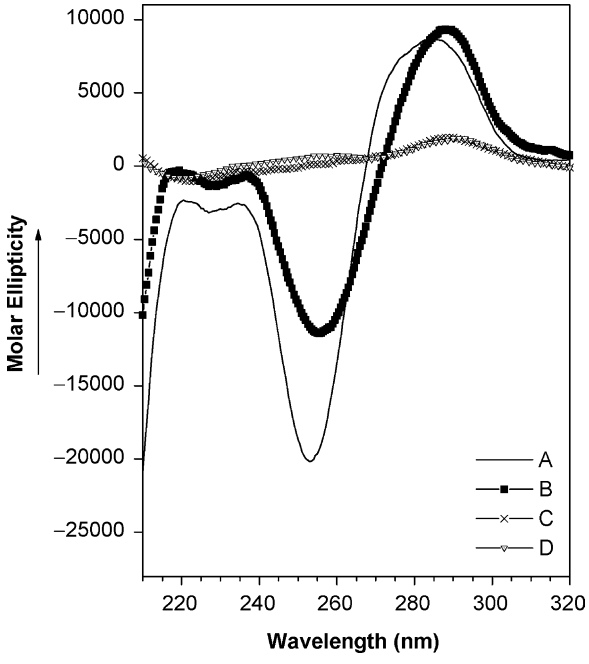

Fig. 2. CD spectra of $d[G(CG)_7]\cdot d[C(GC)_7]$, 60 μM in phosphate, at different pH values. (A) 7.5, (B) 5.0, (C) 3.0, (D) 1.5.

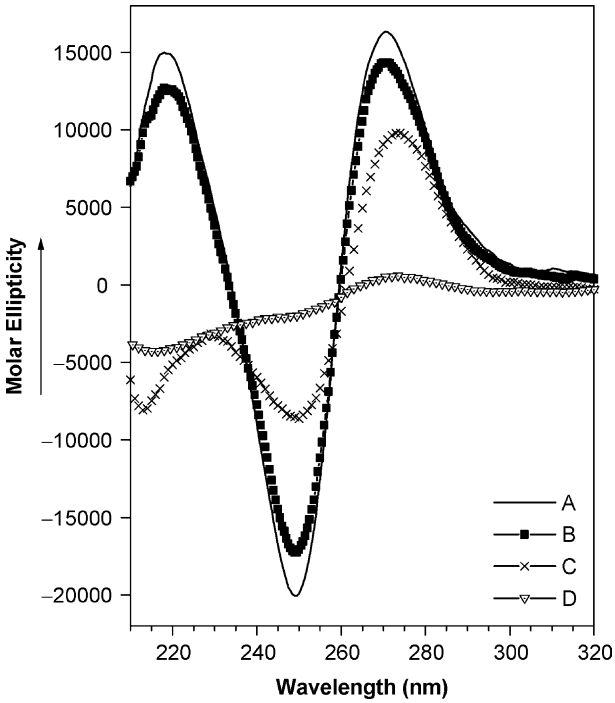

Fig. 3. CD spectra of $d[A(TA)_7]\cdot d[T(AT)_7]$, 60 μM in phosphate, at different pH values. (A) 7.5, (B) 5.0, (C) 3.0, (D) 1.5.

3.2. Effect of the interaction with the spermine aminooxy analogue AOSPM

The CD spectra of the oligonucleotides in the presence of AOSMP, at pH 7.5 and 5.0, are shown in Fig. 5. Dramatic differences were observed between both series of spectra. At physiological pH, the spectra of $d[G(CG)_7]\cdot d[C(GC)_7]$ showed negligible changes when adding AOSPM. On the contrary, the spectra of the A–T oligonucleotide exhibited ellipticity decrease in the band at 271 nm, which shifted downwards by 4 nm. At pH 5.0, all the CD bands of $d[A(TA)_7]\cdot d[T(AT)_7]$ exhibited hypochromism in the presence of this aminooxy polyamine. In addition, the spectra showed two isodicroic points, at 235 and 259 nm, which indicates the presence of a conformational equilibrium for this oligonucleotide involving three different species in solution. CD spectra of $d[G(CG)_7]\cdot d[C(GC)_7]$-AOSPM solutions also exhibited an isodicroic point at 245 nm; the positive band at 288 nm showed intensity decrease and shifted upwards by 8 nm, while the band at 228 nm changed its

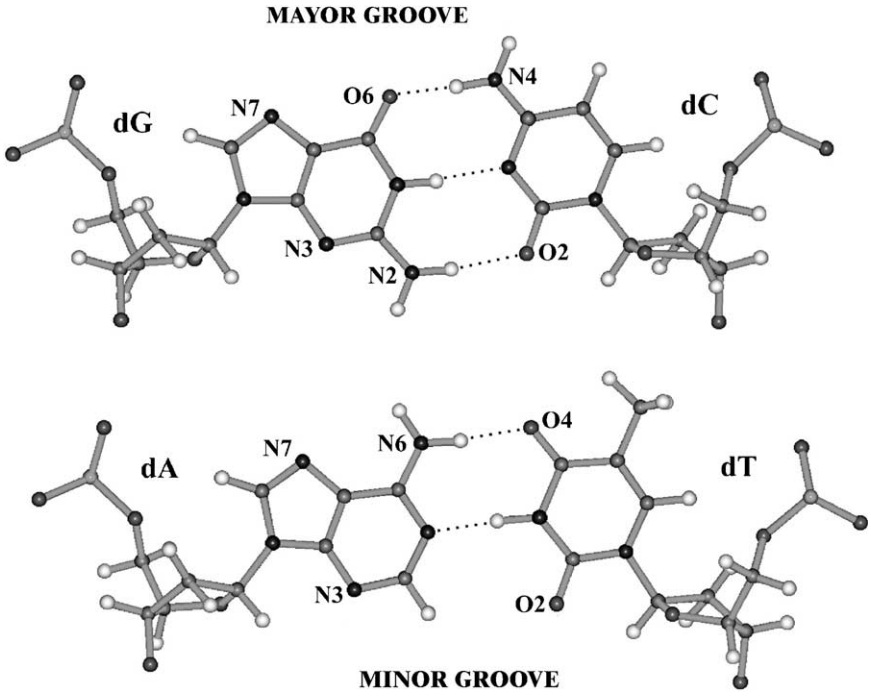

Fig. 4. The Watson–Crick bonding of A–T and G–C base pairs.

sign from positive to negative when the AOSPM concentration reached 1000 μM.

The 300–250 nm region is relevant in relation to structural changes on DNA and oligonucleotides. It has been demonstrated that there is an inverse relationship between the intensity of the bands in this region and the angle formed by two adjacent base pairs of the nucleotic sequence, which is known as the winding angle [23]. The intensity decrease observed for the 271 nm CD band of $d[A(TA)_7]\cdot d[T(AT)_7]$, at pH 7.5, in the presence of AOSPM would be therefore related with an increase of the oligonucleotide winding angle. This fact can be explained from the ability of the AOSPM molecules to preferently bind with the major groove of A–T motifs, as suggested from experimental and theoretical studies [24]. It has been also suggested that spermine binds by the two grooves in A–T enriched sequences [16], thus favouring denaturation during nucleic acid initiations processes, in contrast to AOSPM. This difference could provide useful information about the molecular bases of the growth-promoting functions of spermine. At pH 5.0, CD spectral changes upon AOSPM addition are similar to those observed upon pH lowering, thus suggesting a double-strand destabilization induced by the AOSPM molecules. The different response to AOSPM addition at different pH values could be related with the protonation of the aminooxy group. Since its pK_a is about 4.5, AOSPM has a molecular charge near +4 at pH 5.0, so that it would be able to provoke DNA precipitation into solid particles, as observed for other tetracations [25].

The binding model proposed for AOSPM with G–C rich regions involves a similar interaction by the two DNA grooves [24]. On the other hand, the G–C base pairing is stronger than the A–T one because of the presence of an extra hydrogen bond. These facts can explain why the CD spectrum of $d[G(CG)_7]\cdot d[C(GC)_7]$ remains almost unchanged when adding AOSPM at a physiological pH, contrarily to what happened with the A–T oligonucleotide. The greater positive charge this aminooxy polyamine has at pH 5.0 induced critical changes on the CD spectrum of the G–C oligonucleotide. The appearing of an isodicroic point means that two different conformations are present in the solutions. The observed features are similar to those observed for the decamer $d(GC)_5$ in the presence of trifluorethanol [26], which is considered as inductor of a conformational change from the right-handed B-DNA to the left-handed Z-DNA secondary structures. In consequence, we postulate this oligonucleotide, in the presence of AOSPM, is involved in a conformational equilibrium between these two structures, which explains the existence of the aforementioned isodicroic point.

3.3. Effect of the interaction with the spermidine aminooxy analogues AOEPUT and APAPA

The CD spectra of the oligonucleotides in the presence of AOEPUT and APAPA are shown in Figs. 6 and 7, respectively. As can be seen, spectral changes upon aminooxy polyamine addition only appeared for solutions at acidic pH, it is to say, when most of the aminooxy groups are protonated.

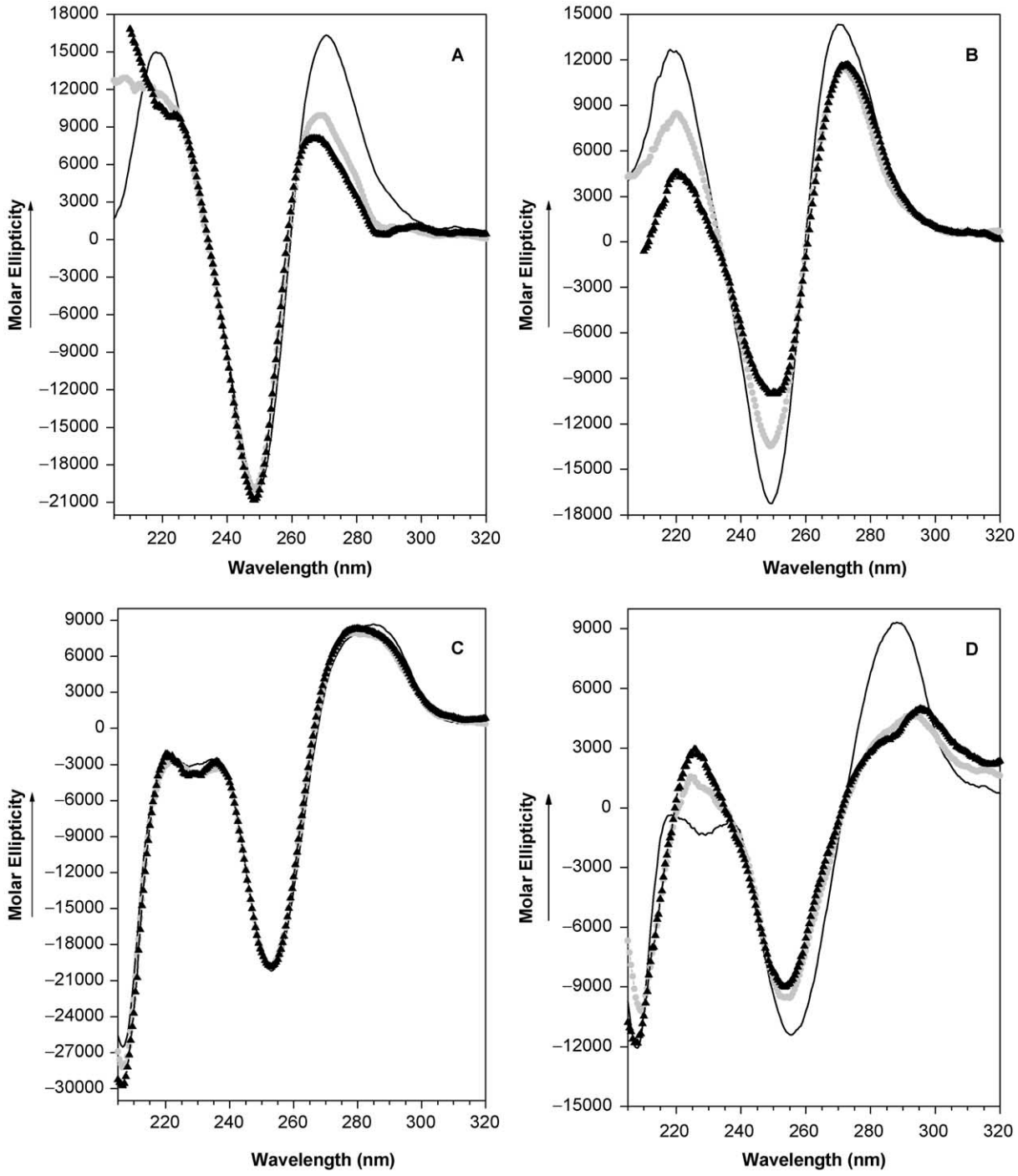

Fig. 5. CD spectra of the A–T and G–C oligonucleotides, 60 μM in phosphate, in the presence of AOSPM. (A) A–T, pH 7.5; (B) A–T, pH 5.0; (C) G–C, pH 7.5; G–C, pH 5.0. The AOSPM concentrations are: 0 (—), 250 (●●●, grey) and 1000 μM (▲▲▲, black).

These features are similar to those observed for AOSPM-$d[A(TA)_7]\cdot d[T(AT)_7]$ solutions, thus indicating double-strand destabilization induced by AOEPUT and APAPA. Spectra at physiological pH did not evidence changes on the macromolecular structure of the A–T oligonucleotide. Previous works have demonstrated that dications are not able to induce macromolecular changes on both long and short DNA changes [27–29], which agrees with our present results.

Concerning spectra of $d[G(CG)_7]\cdot d[C(GC)_7]$ solutions, they did not exhibit inversion of the band at 228 nm, so the presence of Z-DNA conformation induced by AOEPUT or APAPA are not undoubtedly demonstrated. In our opinion, the observed CD features are compatible with an oligonucleotide denaturation favoured by the partial protonation of the cytosine-N3 position, and the subsequent relaxation of the inter-strand hydrogen bonding, at acidic pH.

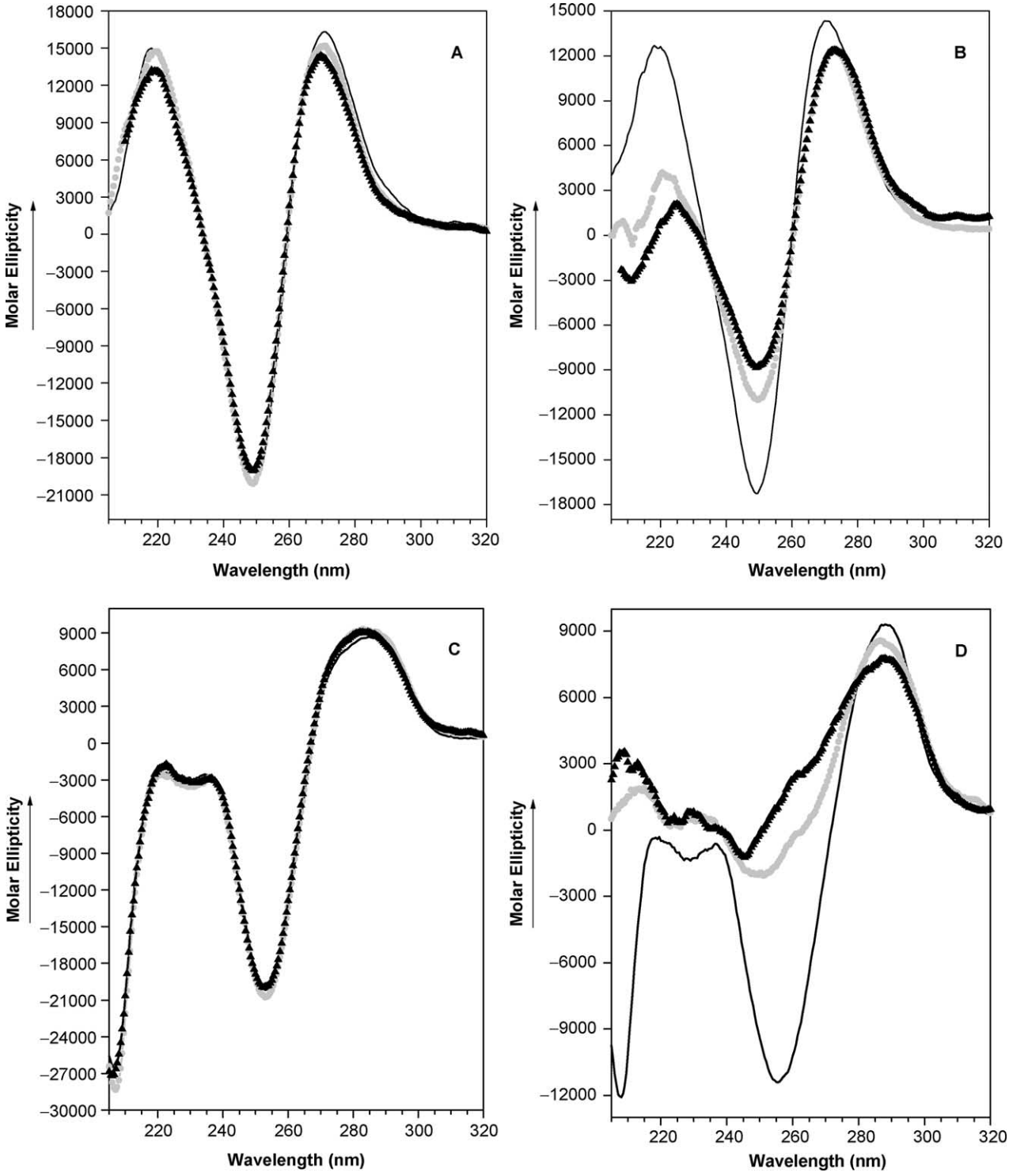

Fig. 6. CD spectra of the A–T and G–C oligonucleotides, 60 μM in phosphate, in the presence of AOEPUT. (A) A–T, pH 7.5; (B) A–T, pH 5.0; (C) G–C, pH 7.5; G–C, pH 5.0. The AOEPUT concentrations are: 0 (—), 250 (●●●, grey) and 1000 μM (▲▲▲, black).

It is worthwhile to point out the different behaviour showed by the negative feature at 206 nm of $d[A(TA)_7]\cdot d[T(AT)_7]$ upon addition of these spermidine analogues. This band becomes positive with AOEPUT, while remains as negative with APAPA. Both molecules are isosteric to spermidine. However, the aminooxy group is located at the N1 position of spermidine in AOEPUT, while it is at the N8 position of spermidine in APAPA. The observed difference supports that these positions could have non-equivalent contributions in the interaction of this biogenic polyamine with DNA.

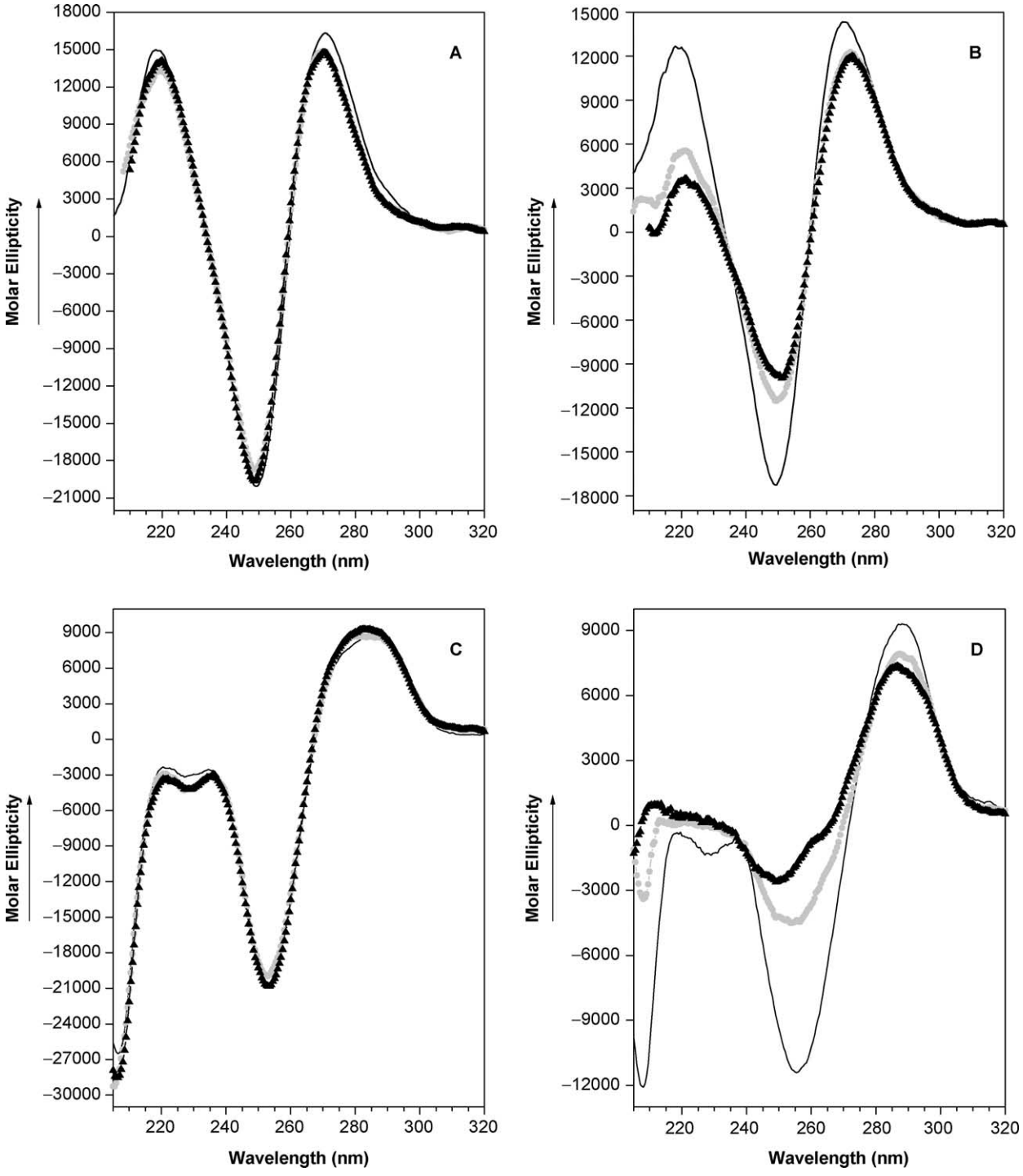

Fig. 7. CD spectra of the A–T and G–C oligonucleotides, 60 μM in phosphate, in the presence of APAPA. (A) A–T, pH 7.5; (B) A–T, pH 5.0; (C) G–C, pH 7.5; G–C, pH 5.0. The APAPA concentrations are: 0 (—), 250 (●●●, grey) and 1000 μM (▲▲▲, black).

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