An A-form of poly[d(A-C)]·poly[d(G-T)] induced by mercury (II) as studied by UV and FTIR spectroscopies

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Abstract The conformational changes of poly[d(A-C)]·poly[d(G-T)] induced by $Hg(ClO_4)_2$ in aqueous solution have been studied using UV absorption and fourth derivative spectrophotometries, and FTIR spectroscopy. The UV absorption and fourth derivative spectra reflect changes in the polynucleotide stacking interactions as a result of the metal–polynucleotide interaction. The fourth derivative spectra do not indicate a Z-form either at low or at high metal-to-polynucleotide ratios. Furthermore, the infrared spectrum at high metal-to-polynucleotide ratio ($r = 1.2$; $r = [Hg(ClO_4)_2]/[\text{nucleotide}]$ molar ratio) has the main features of an A-form, in contrast with previous CD studies which proposed that the polynucleotide adopts a Z-form under these conditions. The nature of a different conformation of the polynucleotide induced at low r-ratios ($r \leq 0.2$) is discussed.

Key words: A-DNA; Mercury (II); FTIR spectroscopy; UV fourth derivative spectrophotometry; poly[d(A-C)]·poly[d(G-T)]

1. Introduction

The $(dA\text{-}dC)_n \cdot (dG\text{-}dT)_n$ sequence is found repeatedly in the eukaryotic genome [1]. There is evidence that this sequence can regulate transcription [2–4] among other effects on cellular processes. The double-stranded synthetic polynucleotide poly[d(A-C)]·poly[d(G-T)] is a good model for physicochemical studies that can provide further insight in to the structural requirements for the functionality of these naturally occurring repeats. This polynucleotide has been shown to be ultrapolymorphic since it can adopt B, A, Z and Z* conformations in solution [5]. On the other hand, Hg(II) has been shown to induce topological changes in native calf thymus DNA [6] and in several synthetic polynucleotides [7–9]. In particular, the Hg(II)-induced structural transitions of poly[d(A-C)]·poly[d(G-T)] have been studied by means of CD spectroscopy [8]. These conformational changes have been suggested to be related to the genotoxic damage caused by inorganic mercury in eukaryotes [8]. However, this latter CD study did not allow the observed conformations to be unambigously assigned due to the fact that CD spectroscopy can yield artifactual information regarding structure assignments [10].

In the present study we have used the fourth derivative of the UV absorption spectra to analyze the conformational changes of the polynucleotide induced by $Hg(ClO_4)_2$. We have previously shown that the fourth derivative technique can provide more detailed information about both polynucleotide structure [11] and polynucleotide conformational transitions ([12], and unpublished results) than classical absorption spectrophotometry. In addition, FTIR spectroscopy, a very well-established technique in the study of polynucleotide structure [13], has been used as a complementary tool to gain further insight into the metal-polynucleotide interaction. The results obtained by combining these two spectroscopic methods indicate that the polynucleotide exists as a right-handed B-form in the absence of $Hg(ClO_4)_2$, and that it adopts an A-form at high ($r = 1.2$) metal-to-polynucleotide ratios. The nature of the different conformations which are detected at low r ratios ($t \leq 0.2$) is discussed.

2. Materials and methods

Poly[d(A-C)]·poly[d(G-T)], from Boehringer, was the generous gift of Dr. W. Guschlbauer (Gif-sur-Yvette, France). $Hg(ClO_4)_2 \cdot 3H_2O$ was from Aldrich. All other chemicals used were of reagent grade.

The lyophilized polymer was dissolved in distilled water and dialyzed in a stepwise procedure, first against 0.5 M NaCl, 10 mM CDTA, pH 8.0, which eliminates most of the metal ions [14]. The final treatment of the polymer was with 5 mM Tris-HCl, 50 mM NaCl, pH 7.8 (buffer solution). A mercuric perchlorate stock solution of 0.1 M was prepared for each experiment by dissolving the approriate amount of the salt in distilled water; no formation of precipitate was detected during the experiment. Small volumes of this solution were added to the polynucleotide solution in order to obtain the desired metal-to-polynucleotide ratio. Mercury concentrations are expressed in terms of the normalized quantity r, where $r = [Hg(ClO_4)_2]/[\text{nucleotide}]$, i.e. the number of moles of mercury added per mole of nucleotide. The brackets, as usual, denote molar concentrations. For the determination of the polynucleotide concentration the extinction coefficient, at 260 nm, was taken to be 6500 $M^{-1} \cdot cm^{-1}$ [15].

UV absorption spectra were obtained with a 320 Perkin-Elmer spectrophotometer. Absorption spectra were the result of 4 scans and were smoothed and derivated as previously described in detail [11]. Infrared spectra were obtained with a Mattson-Polaris FTIR spectrometer in aqueous solution at a resolution of 2 cm^{-1}. Samples were placed in an infrared cell provided with ZnSe windows with a pathlength of 25 μm, set using a Teflon spacer. The concentration of the polynucleotide used for the FTIR experiments, on a nucleotide basis, was higher (typically 15 mM in all infrared spectra) than the one used for UV spectroscopy (typically 170 μM), since this is an intrinsic requirement of the technique, but the r ratios used were the same in both cases. Infrared spectra were recorded at different times to control the kinetics of the polynucleotide conformational transitions and to ensure that no changes in the

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Abbreviations: UV, ultraviolet; FTIR, Fourier-transform infrared spectroscopy; CD, circular dichroism; CDTA, (+)-*trans*-1,2-diamino-cyclohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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spectra were occurring, at the different r ratios, with time. All spectra were obtained at a temperature of 25°C.

All manipulations involving Hg(II) compounds were performed adopting safety measures already described [16].

3. Results and discussion

Fig. 1 shows the absorption spectra of poly[d(A-C)]·poly[d(G-T)] in buffer solution at different r ratios. The final overall changes accompanying $Hg(ClO_4)_2$ addition are a red-shift of the absorption maximum and hyperchromism in the 270–310 nm region. At low r values ($r < 0.2$) the absorption UV spectrum undergoes minor changes. At $r = 0.2$ the absorption UV spectrum shows no shift in the absorption maximum and a small hyperchromism, which could be reflecting a small degree of unstaking of the bases in the polynucleotide as a result of the metal interaction. At a high r ratio ($r = 1.2$) a red shift of the absorption maximum is detected from 260 to 263 nm, as well as a more pronounced hyperchromism in the 270–310 nm region. The fourth derivative spectrum suffers only minor changes at $r = 0.2$ (Fig. 2), indicating that specific stacking interactions are hardly perturbed by the metal addition in the low metal-to-polynucleotide r range. The main change observed in the fourth derivative UV spectrum (Fig. 2) upon an increase in mercuric perchlorate concentration is an increase in the intensity of the peak located at 293 nm (for $r > 0.2$). A slight but significant red-shift of this peak to 293.8 nm can also be detected. Interestingly, a fourth derivative peak at 293 nm has also been observed in the spectrum of the A-form of poly[d(A-T)]·poly[d(A-T)] induced by ethanol [17]. The observed changes in the amplitude and wavelength of this peak could be related to changes in the stacking interactions occurring during the Hg(II)-induced conformational transition. These changes in the stacking interactions must be related, among other factors, to changes in the inclination of the bases. It has been recently shown that, in fact, the bases in poly[d(A-C)]·poly[d(G-T)] are inclined from the perpendicular to the helix axis in the B-form, and that a transition to the A-form induced by trifluoroethanol results in an even larger base inclination [15]. Thus, the changes observed in the long wavelength region of the derivative spectra, namely those associated with the 293 nm peak, could be a result of the specific structural features of this polynucleotide and the specific conformational transitions induced by Hg(II). No significant fourth derivative peaks are detected at longer wavelengths than that of the 293 nm peak, in the range of r varying from 0 to 2, whereas we have already shown that Z-forms of synthetic polynucleotides are characterized by the appearance of new fourth derivative peaks in the 295–300 nm region ([12,18] and unpublished results).

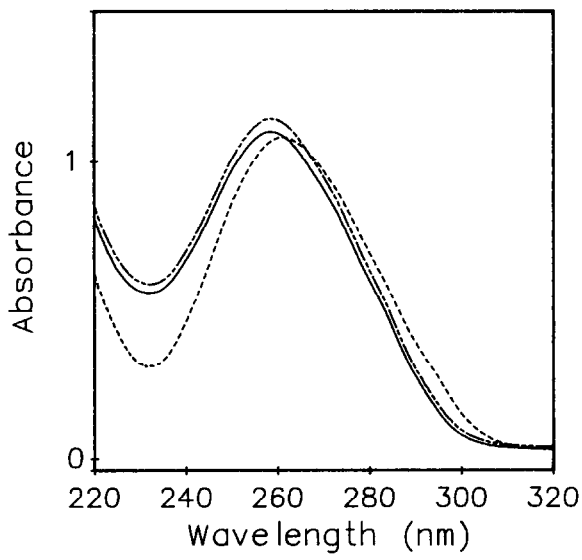

Fig. 1. UV absorption spectra of poly[d(A-C)]·poly[d(G-T)] in 5 mM Tris-HCl, 50 mM NaCl, pH 7.8, at the following r values (r = [Hg(ClO4)2]/[nucleotide], molar ratio): —, $r = 0$; - - -, $r = 0.2$; - - -, $r = 1.2$. Polynucleotide concentration 170 μM.

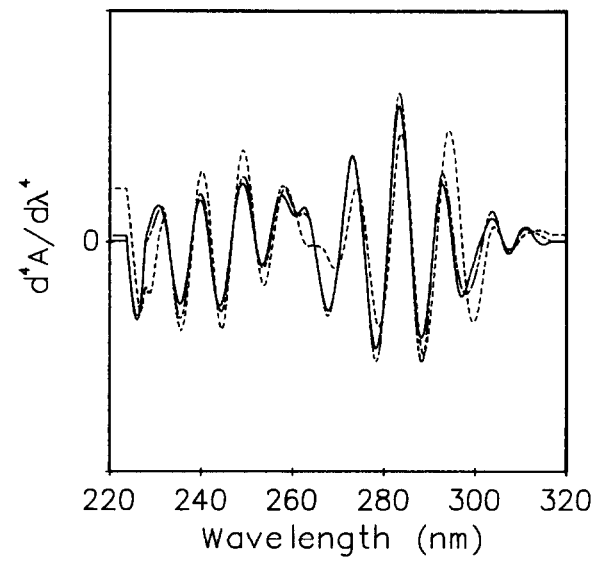

Fig. 2. Fourth derivative of the absorption spectra in Figure 1. —, $r = 0$; - - -, $r = 0.2$; - - -, $r = 1.2$.

Fig. 3 shows the infrared spectra at the same ratios as in the previous figures. The spectrum at $r = 0$ (Fig. 3a) reflects a classical B-conformation with bands at 1224, 1281, 1299 and 1236 cm^{-1}. The band at 1224 cm^{-1} corresponds to the antisymmetric phosphate stretching vibration, as PO_2^-, of the B-family helices [19–21]. The spectrum at $r = 0.2$ (Fig. 3b) shows bands at 1224, 1280, 1302 and 1323 cm^{-1} and shoulders at 1208 and 1253 cm^{-1}. This spectrum has some characteristics of a C- or a D-form [22,23]. The shoulder at 1208 cm^{-1}, for example, has been observed in the D-form of poly[d(A-T)]·poly[d(A-T)] [23]. A band at 1208 cm^{-1} has also been detected in the spectrum of DNA interacting with daunomycin and assigned to hydrogen-bonding between the drug and the polynucleotide phosphate [24]. With regard to this band at 1208 cm^{-1} this could also arise from a splitting of the phosphate band at 1224 cm^{-1} observed in the spectrum at $r = 0$. This change and also the band at 1323 cm^{-1} could be associated with a left-handed conformation. However, our fourth derivative spectra do not show the appearance of any new peak in the 295–300 nm region which is always observed in fourth derivative spectra of Z-forms of different polynucleotides ([12,18], and unpublished results). Thus, in view of these results the possibility that poly[d(A-C)]·poly[d(G-T)] adopts a left-handed conformation at low r ratios can not be completely ruled out. If this is the case, however, this confor-

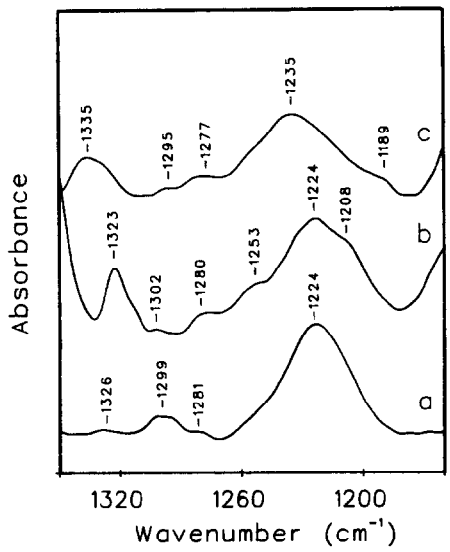

Fig. 3. FTIR spectra of poly[d(A-C)]·poly[d(G-T)] in aqueous solution; same conditions as in Fig. 1. (a) $r = 0$; (b) $r = 0.2$; (c) $r = 1.2$. Polynucleotide concentration 15 mM.

mation is likely to be different from that of canonical Z-DNA which would have to show the characteristic antisymmetric phosphate band at 1215 cm^{-1}, among other characteristic features [19–21], not observed in our spectrum. The spectrum at a high r value ($r = 1.2$) (Fig. 3c) shows typical changes that have been associated with a transition to an A-conformation. The phosphate band at 1224 cm^{-1} shifts to 1235 cm^{-1} and the band at 1281 cm^{-1} shifts to around 1277 cm^{-1}. There is also a shoulder at 1189 cm^{-1} and a band at 1335 cm^{-1}. We obtained very similar results for the A-form of poly[d(n^2a-T)]·poly[n^2a-T)] (n, amino) [25,26]. Also, a band at 1215 cm^{-1} would be expected in the case of a left-handed conformation of the polymer [19–21]. The infrared spectra are, thus, indicative of an A-conformation for poly[d(A-C)]·poly[d(G-T)] at high r ratios.

The results here reported are in disagreement with a CD study previously reported [8], in which the negative band in the 280–290 nm region was interpreted as reflecting a left-handed structure for the polymer at $r > 0.2$. We have recently shown that polynucleotide CD spectra showing an inverted CD band at around 280 nm are not reflecting a left-handed conformation but rather an A-conformation in the case of the conformational transition of poly[d(n^2A-T)]·poly[d(n^2A-T)] induced by polyamines [25]. Thus, the 220–320 nm region can yield artifacts regarding nucleic acid structure in some cases [27]. Several studies have stressed the importance of analyzing the region below 220 nm (vacuum ultraviolet) which has been demonstrated to be more reliable and to show a better correspondence with the actual secondary structures adopted by nucleic acids in solution [14,28]. This is particularly important in the case of the polynucleotide used in this study which is highly polymorphic [5,14].

Moreover, a careful analysis of the reported CD spectra [8] reveals the following aspects: (i) CD spectra of Z-forms of poly[d(A-C)]·poly[d(G-T)] induced by 4.0 M NaCl, 40 mM $NiCl_2$ [20], by polyamines [29] and divalent cations like Co^{2+} or Ni^{2+} [14], show a clear positive band in the 260–270 nm region which is not observed in the CD study [8]; (ii) the negative band observed around 210 nm, attributed in the abovementioned CD study to a left-handed conformation [8], is mostly observed in the case of the A-form of the polymer [14,15], whereas a negative band characteristic of the left-handed Z conformation is instead detected at lower wavelengths, at around 200 nm [14,15].

In summary, the UV and FTIR results herein reported indicate that poly[d(A-C)]·poly[d(G-T)] adopts an A-like conformation at high r ratios ($r = [Hg(ClO_4)_2]/[\text{nucleotide}]$). A different conformation is detected at low r ratios. If this conformation corresponded to Z-DNA, the appearance of new fourth derivative peaks in the 295–300 nm region would be expected ([12,18], and unpublished results) which is not observed. The possibility of a left-handed conformation for the Hg(II)-induced conformation of poly[d(A-C)]·poly[d(G-T)] at low r ratios can not be ruled out. If this is the case, however, this would probably be a non-B-DNA conformation significantly different from that of canonical Z-DNA, as has been proposed recently [30].

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