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Conformational changes of poly(dG-dC) . poly(dG-dC) modified by the carcinogen N-acetoxy-N-acetyl-2-aminofluorene

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ABSTRACT

Poly(dG-dC).poly(dG-dC) was modified by the reaction with N-acetoxy-N-acetyl-2-aminofluorene. The conformations of poly(dG-dC).poly(dG-dC) and of poly d(G-C)AAF were studied by circular dichroism under various experimental conditions. In 95 % ethanol, the two polynucleotides adopt the A-form. In 3.9 M LiCl, the transition B-form-C-form is observed with poly(dG-dC).poly (dG-dC) but not with poly d(G-C)AAF. In 1 mM phosphate buffer, poly d(G-C)AAF behaves as a mixture of B- and Z-form, the relative percentages depending upon the amounts of modified bases. The percentage of Z-form is decreased by addition of EDTA and is increased by addition of Mg^{++}. Spermine favors the Z-form in modified and unmodified polynucleotides. No defect in the double helix of poly d(G-C)AAF is detected by S_I endonuclease.

INTRODUCTION

The covalent binding of a chemical carcinogen to DNA can have several effects on the conformation of DNA. These effects will depend on numerous factors such as the nature of the modified bases, the base sequence adjacent to the modified bases, the experimental conditions (temperature, ionic strength..). There are several lines of evidences for a local denaturation of DNA due to the covalent binding of acetylaminofluorene residues to the C(8) of guanine residues (general reviews 1,2). On the other hand, we have recently reported[3] that the covalent binding of AAF residues to guanine residues in poly(dG-dC).poly(dG-dC) induces the transition from the B-form to the Z- or a Z-like form, even in low salt concentration. The consequences are that in modified regions, there is no local denaturation, the modified guanines are still paired with cytosine and AAF-residues are outside the double helix. These conformational changes might be of importance in tumorogenic process and it seemed to us of interest to study in more details the conformational changes induced in poly(dG-dC).poly(dG-dC) by the binding of the carcinogen. In the present paper, we report some more results on the parameters which govern the transition B-form - Z-form in AAF modified poly(dG-dC).poly(dG-dC).

Moreover, we show that the modified polynucleotide can adopt the A-form but not the C-form in experimental conditions where the transitions are observed with poly(dG-dC).poly(dG-dC).

MATERIALS AND METHODS

Poly(dG-dC).poly(dG-dC) was purchased from P.L. Biochemicals. It was purified by treatment with phenol and then successively dialyzed against 0.5 M NaCl, 5 mM Tris-HCl pH 7.5, 0.1 mM EDTA during 24 hours, against the same solution without EDTA during 24 hours, against 100 mM NaCl, against 10 mM NaCl and exhaustively against 1 mM phosphate buffer pH 7.5.

The reaction between poly(dG-dC).poly(dG-dC) and N-acetoxy-N-acetyl-2-aminofluorene was performed as already described [3]. The percentages of modified bases were deduced from ultraviolet absorption spectra. We will write poly d(G-C)AAF (10 %) for a modified sample having 10 % modified bases. The sedimentation coefficients in 100 mM NaCl, 5 mM Tris-HCl pH 7.5 were 9.7 S for poly(dG-dC).poly(dG-dC), 11.2 S for poly d(G-C)AAF (4 %) and 11.6 S for poly d(G-C)AAF (7.5 %).

All the salts were analytical grade. S_I endonuclease from *Aspergillus oryzae* was purchased from Boehringer Mannheim.

Ultraviolet absorption and circular dichroism spectra were recorded with a Cary 210 spectrophotometer and a Roussel Jouan III dichrograph, respectively. The circular dichroism (CD) results are reported as $\Delta A = A_L - A_R$, ΔA being the difference in absorption between left and right polarized light at wavelength λ. The results are given in ΔA and not in $\Delta\varepsilon$ $M^{-1}cm^{-1}$ because fluorene and nucleotide residues can participate to the CD signal (see discussion). The optical density at 260 nm of all the solutions was 0.4.

RESULTS

Effects of solvent

The conformations of poly(dG-dC).poly(dG-dC) and poly d(G-C)AAF have been studied by circular dichroism in various media. The spectra are shown in fifure 1.

In 4 M $NaClO_4$ and 1.2 M $MgCl_2$, the spectra of poly(dG-dC).poly(dG-dC) are similar in shape (there are some differences in the intensities of the bands) and are almost an inversion of the spectrum in low salt concentration as already reported in the literature [4]. In these two salts, at high concentration, the CD spectra of poly d(G-C)AAF and of poly(dG-dC).poly(dG-dC) are almost the same respectively.

In 95 % ethanol, the spectra of poly(dG-dC).poly(dG-dC) and of poly d(G-C)

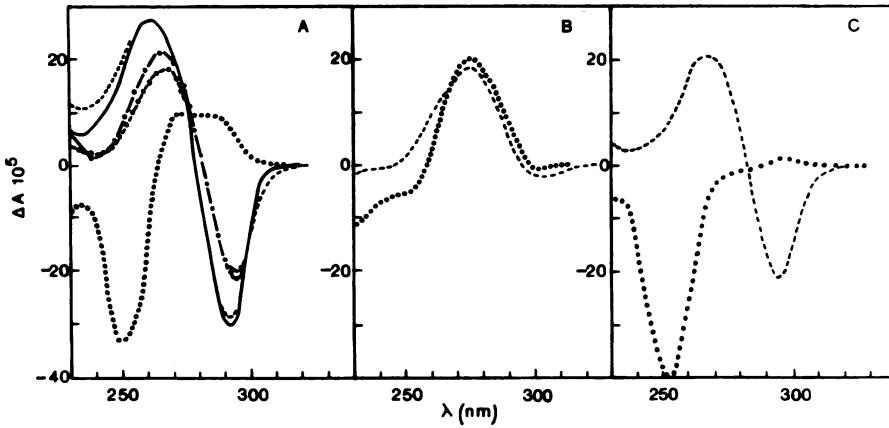

Fig. 1 - Circular dichroism of poly(dG-dC).poly(dG-dC) and of poly d(G-C)AAF (10.5 %).
A) Poly(dG-dC).poly(dG-dC) in 1 mM phosphate buffer pH 7.5 (•••), in 1.2 M $MgCl_2$, 5 mM Tris-HCl pH 7.5 (-•-•-), in 4 M $NaClO_4$, 5 mM Tris-HCl pH 7.5 (——).
Poly d(G-C)AAF in 1.2 M $MgCl_2$, 5 mM Tris-HCl pH 7.5 (—•—), in 2 M $NaClO_4$, 5 mM Tris-HCl pH 7.5 (---).
B) Poly(dG-dC).poly(dG-dC) (•••), poly d(G-C)AAF (---) in ethanol 1 mM phosphate buffer (95:5, v/v).
C) Poly(dG-dC).poly(dG-dC) (•••), poly d(G-C)AAF (---) in 3.8 M LiCl.
The absorbances at 260 nm of all the solutions were 0.4, Temperature 25°C.

AAF are almost the same. There is a very weak negative band and then an intense positive band centered at 275 nm.

In 3.8 M LiCl, the CD spectrum of poly(dG-dC).poly(dG-dC) is quite different from the spectrum in 4 M $NaClO_4$. There is a very weak positive band and then an intense negative band centered at about 253 nm. These changes are not observed with poly d(G-C)AAF. The spectra in 3.8 M LiCl and in 2 M $NaClO_4$ are identical.

Z-form

In low salt concentration, the conformation of poly(dG-dC).poly(dG-dC) is the B-form. In the same experimental conditions, we have shown that the conformation of poly d(G-C)AAF (8.5 %) was partly the Z-form or a Z-like form[3]. In 1 mM phosphate buffer pH 7.5, the CD spectra of poly d(G-C)AAF samples having various percentages of modified bases were recorded. In all the cases, within the experimental errors, the CD differences spectra (poly d(G-C)AAF - poly(dG-dC).poly(dG-dC), in 1 mM phosphate buffer) have the same shape as the CD difference spectra (poly(dG-dC).poly(dG-dC) in 4 M $NaClO_4$ - poly(dG-dC).

poly(dG-dC) in 1 mM phosphate buffer) but the intensities of the bands are different (the results are similar to those shown in figure 4, ref. 3). The variation of ΔA_{290} as a function of the percentage of modified bases is presented in figure 2. As a first approximation this variation can be accounted by a straight line (except for low percentages of substitution). Linear variations were also observed at different wavelengths. Assuming that poly d(G-C) AAF is either in B-form or in Z-form, one can easily calculate the percent of Z-form from these experiments.

In low salt concentration, the CD signal of poly d(G-C)AAF was sensitive to the addition of EDTA (concentration 0.2 mM). Again, the CD difference spectra have the same shape as that relative to poly(dG-dC).poly(dG-dC) (high salt-low salt) but the intensities are different. The variation of ΔA_{290} as a function of the percentage of modified bases is linear as shown in figure 2. The smaller values of ΔA_{290} indicate that the amount of Z-form is reduced by addition of EDTA. Na citrate and Na perchlorate can also decrease the amount of Z-form but they are less efficient than EDTA (Figure 3).

Addition of divalent ions has a reverse effect. The shape of the titration curve depends upon the percentage of modified bases in poly d(G-C)AAF as shown in figure 4. For a sample having 5 % modified bases, the curve is biphasic. The first transition occurs in a concentration range 0.1 mM-1 mM $MgCl_2$

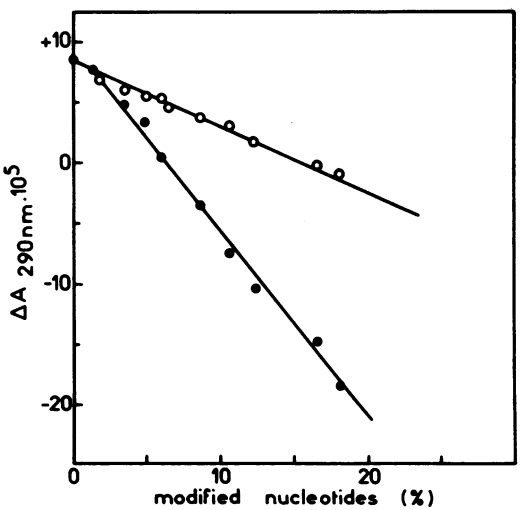

Fig. 2 - Circular dichroism. Variation of ΔA_{290} of poly d(G-C)AAF as a function of the percentage of modified bases, (● ●) in 1 mM phosphate buffer pH 7.5, (o o) same buffer plus 0.2 mM EDTA. Temperature 25°C.

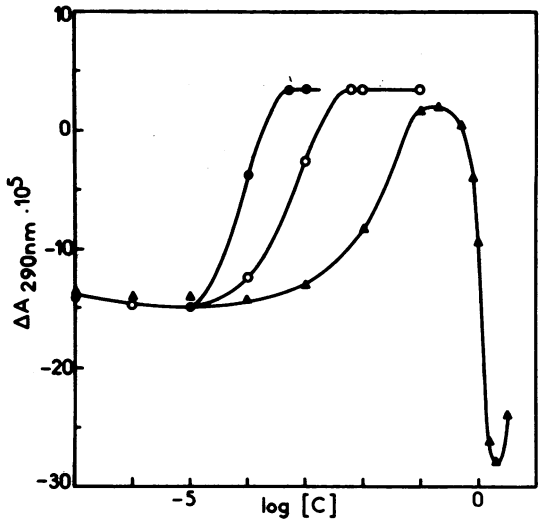

Fig. 3 - Circular dichroism. Variation of ΔA_{290} of poly d(G-C)AAF (10.5 %) as a function of the logarithm of salt concentration. The points (●-●) are relative to the addition of EDTA, (o-o) to the addition of sodium citrate, (▲-▲) to the addition of $NaClO_4$. Temperature 25°C.

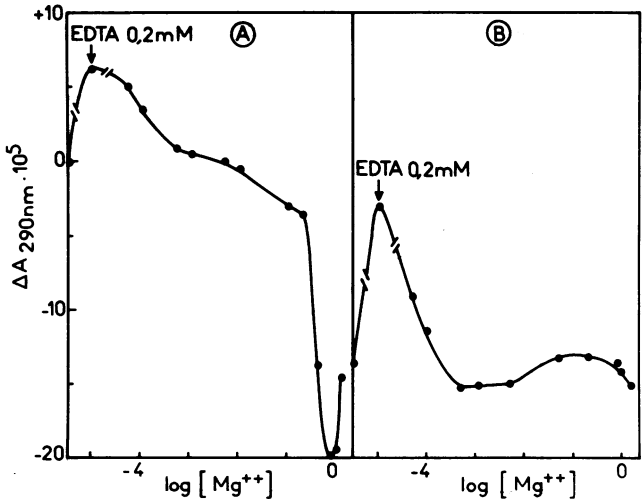

Fig. 4 - Circular dichroism. Variation of ΔA_{290} as a function of the concentration of the logarithm of Mg^{++} concentration. A is relative to poly d(G-C) AAF (5 %), B to poly d(G-C)AAF (10.5 %). ΔA was measured in 1 mM phosphate buffer, then (//) EDTA was added (0.2 mM) and then (//) $MgCl_2$ was added.

and the second one occurs at much higher concentration (in the same range than poly(dG-dC).poly(dG-dC)[5]). A sample having 10 % modified bases presents only one transition at low $MgCl_2$ concentration. In 1 mM $MgCl_2$, this modified polynucleotide is almost completely in the Z-form.

Addition of spermine to poly(dG-dC).poly(dG-dC) or to poly d(G-C)AAF favors the Z-form. For a ratio phosphate over spermine of about 12, in 1 mM NaCl, 1 mM Tris-HCl pH 7.5 at 25°C, the CD spectra of both polynucleotides are similar to the CD spectrum of poly(dG-dC).poly(dG-dC) in high salt concentration (results not shown).

The thermal stability of poly d(G-C)AAF decreases as the amount of modified bases is increased as shown in figure 5. The decrease is of the order of 0.7°C per 1 % of modified bases.

The sensitivity of AAF modified DNA and AAF modified poly(dG-dC).poly (dG-dC) to nuclease S_I from *Aspergillus oryzae* was compared. As already reported in literature (6,17), DNA-AAF and denatured DNA are hydrolyzed by the nuclease (figure 6). In the same conditions, poly(dG-dC).poly(dG-dC) and poly d(G-C)AAF are not hydrolyzed (it was verified by CD that poly d(G-C)AAF was partly in Z-form in the hydrolysis medium).

DISCUSSION

In this work, we have compared the conformation of poly(dG-dC).poly(dG-dC) and of poly d(G-C)AAF mainly by circular dichroism. Several studies have

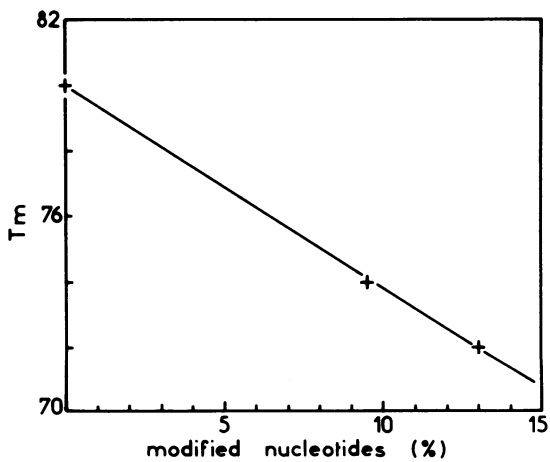

Fig. 5 - Thermal stability of poly d(G-C)AAF. Variation of Tm as a function of the percentage of modified bases. Solvent 1 mM NaCl, 1 mM Tris-HCl pH 7.5, 0.1 mM EDTA.

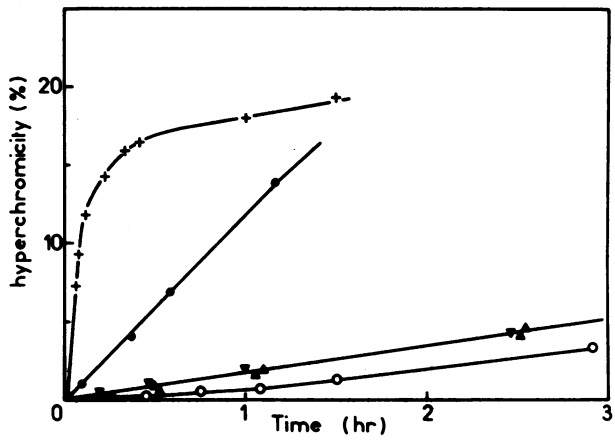

Fig. 6 - Hydrolysis by S_I endonuclease. Hyperchromicity at 260 nm as a function of time. Poly(dG-dC).poly(dG-dC) (Δ), poly d(G-C)AAF (4 %) (▲), poly d(G-C)AAF (7.5 %) (▼), denatured calf thymus DNA (+), native calf thymus DNA (o), native calf thymus DNA-AAF (5.7 %) (●). Solvent 65 mM NaCl, 25 mM sodium acetate pH 4.6, 1 mM $ZnSO_4$. Temperature 37°C.

been already performed on poly(dG-dC).poly(dG-dC)[4,5]. The shapes of the spectra in various media are well established and look like the spectra of natural nucleic acids in known conformations[7-9,19]. On the other hand, in the poly d(G-C)AAF samples we have studied, the optical activity of fluorene chromophores is weak and thus we assume that the conformation of poly d(G-C) AAF can be deduced from circular dichroism results (we are aware that circular dichroism cannot unambiguously prove that a polymer exists in a given form[9]).

The CD spectra of poly d(G-C)AAF and poly(dG-dC).poly(dG-dC) in 95 % EtOH are similar. They are non conservative and typical for double helix RNA-like conformation. Thus AAF residues do not prevent poly d(G-C)AAF to be in A -form.

In 3.8 M LiCl, the CD spectrum of poly(dG-dC).poly(dG-dC) looks like the spectrum of Li-DNA in films (C-form). The spectrum of poly d(G-C)AAF is completely different from the spectrum of poly(dG-dC).poly(dG-dC) and is similar to the one in 4 M $NaClO_4$. Thus in these salt conditions, the conformation of poly d(G-C)AAF is not the C-form. AAF-residues hinder the B → C transition.

In 4 M $NaClO_4$, in 1.2 M $MgCl_2$ and in 50 % ethanol[3], the spectra of modified and unmodified polynucleotides are similar. The two polynucleotides adopt the Z-form or a Z-like-form. The Z-form was first described by Wang et

al.[10]. They studied the structure of the hexanucleotide $(dG\text{-}dG)_3$. This fragment crystallizes as a left handed double helical molecule with Watson-Crick base pairs. The deoxycytidine residues have the anti conformation whereas the deoxyguanosine residues have the syn conformation. According to Wang et al., the high-salt form of poly(dG-dC).poly(dG-dC) is the Z-form. This identification has received further support from a nuclear magnetic resonance study of $(dG\text{-}dC)_8$[11], an X-ray study[12] and an hydrogen exchange study of poly(dG-dC).poly(dG-dC)[13].

We have already shown that in low salt concentration and in absence of ethanol, poly d(G-C)AAF (6.6 %) is partly in Z-form[3]. This was deduced from the comparison of CD difference spectra of poly(dG-dC).poly(dG-dC) (high salt-low salt) and of poly d(G-C)AAF, low salt - poly(dG-dC).poly(dG-dC), low salt. These difference spectra have the same shape but the intensities of the bands are different. Similar results were obtained with poly d(G-C)AAF samples having various percentages of modified bases. In all the cases, the optical activity of AAF residues is small and is negligible as compared to that of poly(dG-dC).poly(dG-dC). Thus, poly d(G-C)AAF behaves as a mixture of poly (dG-dC).poly(dG-dC) in Z-form and B-form. In the Z-form, the guanine residues have the syn conformation and thus the AAF-residues are outside the double helix. This can explain the weak optical activity of these residues. It has been already reported that AAF modified oligonucleotides have an intense optical activity which was due to a strong stacking between the AAF residues and the bases[2,20-22]. It is interesting to note similar alterations on CD spectra of AAF or mitomycin modified poly(dG-dC).poly(dG-dC)[18].

The intensities of CD spectra depend not only on the percentage of modified bases but also on the experimental conditions.

The percentage of Z-form in poly d(G-C)AAF depends upon the amount of modified bases. By linear extrapolation of ΔA_{290} as a function of the percentage of modified bases, in absence of EDTA one finds that poly d(G-C)AAF is completely in Z-form for about 18-25 % modified bases. This value has to be considered with caution. It depends upon the limit value chosen for poly(dG-dC).poly(dG-dC) in Z-form (we recall that ΔA_{290} are different in 1.2 M $MgCl_2$ and 4 M $NaClO_4$). In the CD experiments, the absorbance of all the solutions was 0.4 at 260 nm. The absorbance of AAF residues is not negligible as compared to that of (dG-dC) residues for highly substituted polynucleotides. A correction has to be done in order to get the same nucleotide concentration in all the solutions. This is meaningful since the CD signals of AAF-residues are negligible in comparison with that of (dG-dC) residues. After correction,

the variation of ΔA_{290} as a function of the percentage of modified bases is no more linear (there is a downward curvature, results not shown). One can estimate that poly d(G-C)AAF adopts the Z-form for about 14-20 % modified bases.

Up to this point an important remark has to be done. N-acetoxy-N-acetyl-2-aminofluorene mainly reacts on the C(8) of guanine residues[14]. However some substitutions also occur on the exocyclic amino group of guanine residues but to a much lower extent than on the C(8) (the molar ratio C(8) adduct over N(2) adduct is about equal to 9, R.P.P. Fuchs, personal communication). We do not know whether this adduct, in low amount as compared to the C(8) adduct, can induce the Z-form. It seems likely that the C(8) adducts are responsible for the effects reported in this work but the N^2 adducts cannot be excluded.

The addition of EDTA decreases the percentage of Z-form in poly d(G-C) AAF. Na citrate and $NaClO_4$ produce the same effects but at higher concentrations. An explanation might be that EDTA, Na citrate or $NaClO_4$ remove divalent cations from the polynucleotide. This is supported by the finding that addition of small quantities of $MgCl_2$ has a reverse effect, i.e., an increase of the Z-form. This does not imply that Mg^{++} was present. Some other cations might have the same effect. A systematic study has not yet been done. We recall that before use, poly(dG-dC).poly(dG-dC) was dialyzed against EDTA and that twice-distillated water and first grade salts were used. Nevertheless, very small amounts of Mg^{++} (of the order of the concentration of the polynucleotide, see figure 3) favors the transition B-form → Z-form of poly d(G-C)AAF. The Z-form can also be induced by the additional positively charged molecules like spermine. For a ratio phosphate over spermine of about 12, both polynucleotides are in the Z-form. On the other hand, it has been recently reported that poly(dG-dC).poly(dG-dC) associated with histone octamers does not contain Z-DNA[15].

Poly d(G-C)AAF is thermally less stable than poly(dG-dC).poly(dG-dC). The decrease is about 0.7°C per 1 % of modified bases. This is smaller than the decrease reported for DNA-AAF (1.1°C per 1 % of modified bases,[16]). This decrease does not imply a local denaturation of poly d(G-C)AAF. The thermal stability of poly(dG-dC).poly(dG-dC) in the Z-form is not yet known.

S_I endonuclease from *Aspergillus oryzae* has been already used to show the presence of locally denatured regions in DNA-AAF[6,17]. The kinetics of hydrolysis of two poly d(G-C)AAF samples having 4 and 7.5 % modified bases by endonuclease were measured and compared to native DNA-AAF and denatured DNA. Poly d(G-C)AAF and poly(dG-dC).poly(dG-dC) gave the same results and were almost not hydrolyzed. In the same experimental conditions, native DNA-AAF

(5.7 %) was hydrolyzed but slower than denatured DNA. No defect in the double helix of poly d(G-C)AAF can be detected by S_I nuclease. This is in agreement with the CD results showing that poly d(G-C)AAF is a mixture of B-form and Z-form, the relative amounts of the two forms depending upon the percentage of modified bases.

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