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Spectroscopic studies of (m^5dC-dG)$_3$: thermal stability of B- and Z-forms

Brigitte Hartmann[1], Nguyen Thanh Thuong[1], Jean Pouyet[2], Marius Ptak[1] and Marc Leng[1]

[1]Centre de Biophysique Moléculaire, CNRS, 1A, avenue de la Recherche Scientifique, 45045 Orleans Cedex, and [2]Institut de Biologie Moléculaire et Cellulaire, 15, rue Descartes, 67084 Strasbourg Cedex, France

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ABSTRACT

The hexanucleoside pentaphosphate d(m^5CpGpm^{5}CpGpm^{5}CpG) has been studied in solution by ultra-violet absorption, circular dichroism and ^{31}P nuclear magnetic resonance under various experimental conditions. In 0.2 M $NaClO_4$ at low temperature, an hexamer duplex is formed which has a B or B-like conformation. As the salt concentration is increased, a transition from a B-form to the Z-form occurs and is complete in 3 M $NaClO_4$. In 3 M $NaClO_4$, the behavior of the Z double helix is complex as a function of temperature. The variation of the circular dichroism at 295 nm is biphasic. A first transition occurs over a large range of temperature and corresponds to a conformational change due to a non-cooperative intramolecular process. Ultra-violet absorption and ^{31}P nuclear magnetic resonance show that the new conformation arising from a distortion of the backbone is not similar to that observed in low salt conditions (B-form). At high hexanucleotide concentration, aggregates are formed. The second transition is cooperative and corresponds to the melting of a double stranded helix into single strands.

INTRODUCTION

Immunochemical studies have brought strong evidences that segments of natural DNAs can adopt the Z-conformation. Z-DNA was discovered from an X-ray crystallographic analysis of alternating oligodeoxynucleotides $(dCpdG)_n$ (1-3). It is a left-handed double helix in which the guanine have the *syn* conformation and the cytosine the *anti* conformation. The deoxyguanosine residues have the C3' *endo* conformation and the deoxycytidine the C2' *endo* conformation. The asymmetric unit is a dinucleotide (4). Z-DNA is a strong immunogen (5-6). The antibodies to Z-DNA bind to chromosomes of *Drosophila melanogaster*, *Chironomus thummi* and *Stylonychia mytilus* as visualized by fluorescent staining (7-10). Z-DNA immunoreactivity was also detected in most but not all nuclei of rat cerebellum, liver, kidney and testis (11). In testis, nuclei of spermatogonia were heavily stained whereas the nuclei of spermatocytes, spermatides and sperm remained largely unstained.

Several factors stabilize the Z conformation (general review 12) and among

them, methylation of cytosine residues is very efficient. Behe and Felsenfeld have shown that in 50 mM NaCl plus traces of divalent or polyvalent ions poly (dG-m^5dC).poly(dG-m^5dC) adopts the Z conformation (13).

The hexamer (m^5dC-dG)$_3$ has been crystallized (14). It forms a left-handed Z-DNA helix which is similar to the unmethylated Z-DNA structure with slight modifications due to the methyl groups. In high salt conditions, the hexamer (dC-dG)$_3$ has the left-handed structure as judged by Raman spectroscopy (15) and circular dichroism (16).

In this work, we have studied the conformation of the hexanucleotide pentaphosphate (m^5dC-dG)$_3$. We show by circular dichroism, ultra-violet absorption and ^{31}P NMR that in high salt solution and at low temperature, the hexanucleotide adopts the Z or a Z-like conformation. As the temperature is increased, far below the melting of the double helix, a conformational change due to a cooperative intramolecular process occurs. This new conformation is neither similar to the B conformation observed at low salt concentration nor to the single strand observed at high temperature.

MATERIAL AND METHODS

The synthetic procedure used to prepare the hexadeoxynucleotide pentaphosphate m^5dCpdGpm^{5}dCpdGpm^{5}dCpdG which will write (m^5dCpdG)$_3$ has been already described (17a). Briefly, this synthesis involves preparation of 5-methyldeoxycytidine-3' phosphodiester which is obtained in one step process from 5'-o-di-p-methoxytrityl-N-anisoyl-5 methyl deoxycitidine and by using the mixture methyl-p-chlorophenylphosphorochloridate-1,5-dimethyltetrazolpyridine (17b).

The purity of the hexanucleotide was verified by HPLC (column Hibar RP-18, Merck). Two peaks corresponding to m^5dcytidine and dguanosine were detected after hydrolysis of the hexanucleotide by P_1 nuclease and alkaline phosphatase. There was only one peak for the intact hexanucleotide.

Ultra-violet spectra were recorded on a spectrophotometer Cary 210 and circular dichroism on a dichrograph Jouan-Roussel III. ^{31}P NMR spectra were recorded on a WH 90 Bruker Fourier transform spectrometer operating at 36.43 MHz. In qualitative studies, the spectra were recorded with broad band proton noise decoupling. In the measurements of the relative intensities of the components, the inverse gated decoupling method was used in order to get decoupled spectra without nuclear Overhauser enhancement (nOe). Sedimentation equilibrium experiments were performed in a model E Spinco ultracentrifuge equipped with a digital scanner. Absorbances were measured within $\pm$ 1 10^{-3} unit and distances from the axis were determined within $\pm$ 4 μm (41).

RESULTS

Sedimentation Equilibrium

The molecular weight of the hexanucleotide $(m^5dC\text{-}dG)_3$ has been determined in low and high salt concentrations, at low temperature, by sedimentation equilibrium experiments. The experiments were performed in ^2H_2O and H_2O. The values of partial specific volume $\bar{v}$ of high molecular weight nucleic acids are known but $\bar{v}$ of oligonucleotides are unknown. The results relative to the experiment performed in 3 M $NaClO_4$ at 5°C are shown in figure 1. The variation of Ln A (A being the absorbance) as a function of r^2 is linear. According to the method of Edelstein and Schachman (18), assuming 17 exchangeable protons for the hexanucleotide, one deduces that $\bar{v}$ is equal to 0.64 ml g^{-1} and thus the molecular weight of the sample is 3940 which is almost exactly the expected molecular weight for a duplex $(m^5dC\text{-}dG)_3.(m^5dC\text{-}dG)_3$. It has been verified that for a hexanucleotide concentration up to 10^{-4} M, the variation of Ln A versus r^2 is linear. In this range of concentration, there is no aggregation of the hexanucleotide duplexes. Similar experiments were performed in 0.2 M $NaClO_4$ at 5°, 10° and 15°C. Again, the variation of Ln A versus r^2 were linear (results not shown) and the measured molecular weight is in excellent agreement with the expected molecular weight for the hexanucleotide duplex.

Ultra-violet Absorption

The u.v. spectra of the hexanucleotide in 0.2 M and 3 M $NaClO_4$ at 0°C,

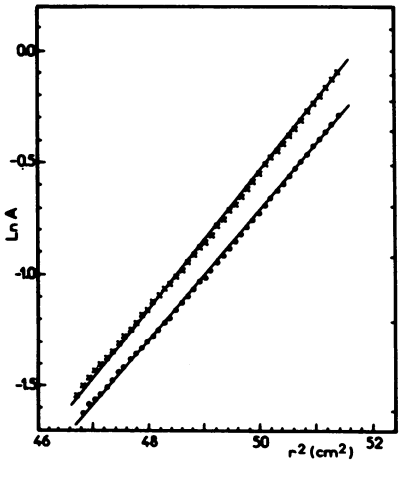

Figure 1 - Sedimentation equilibrium of $(m^5dC\text{-}dG)_3$. Variation of the logarithm of the absorbance at 255 nm as a function of the square of the distance r from the axis of rotation. Solvent 3 M $NaClO_4$, 5 mM Tris-HCl, pH 7.5 in H_2O (x), in ^2H_2O (•). Temperature 5°C. Speed 40161 rpm.

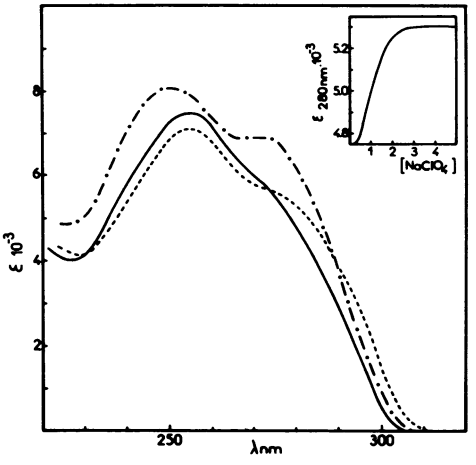

Figure 2 - Ultraviolet absorption of (m^5dCpdG) in 0.2 M $NaClO_4$, 10 mM Tris-HCl at 0°C (——) and at 70°C (—•—), in 3 M $NaClO_4$, 10 mM Tris-HCl at 0°C (---). Inset : variation of ε_{280} as a function of $NaClO_4$ concentration.

are shown in figure 2. These spectra present several similarities with those of poly(dG-m^5dC).poly(dG-m^5dC) (13), i.e., and hyperchromic effect in the range 300-275 nm and a hypochromic effect in the range 270-240 nm as the salt concentration is increased from 0.2 M to 3 M $NaClO_4$. There is an isosbestic point at 271 nm. The midpoint of the transition between these two forms is at about 1.2 M as shown in the inset of figure 2.

The thermal stability of low and high salt forms depends upon the hexanucleotide concentration. The variation of the absorbance A at a given wavelength expressed as $H = (A_{80}-A_T)/A_{80} \times 100$ is represented in figure 3.

In 0.2 M $NaClO_4$, the maximum value of H_{275} is 20 % and is independent of the hexanucleotide concentration. The variation of 1/Tm as a function of Ln c is linear, Tm being the midpoint of the transition and c the hexanucleotide concentration (inset figure 3). The total enthalpy for the oligonucleotide melting deduced from the slope of the straight line representing 1/Tm versus Ln c (19-21) is equal to 60 Kcal $mole^{-1}$.

In 3 M $NaClO_4$, H_{275} increases as the hexanucleotide concentration is raised (from 16 %, c = 2.6 10^{-5} M to 19 %, c = 1.3 10^{-3} M). Nevertheless, the variation of 1/Tm versus Ln c is linear (inset figure 3) and one deduces a value of ΔH equal to 45 Kcal $mole^{-1}$. At 80°C, the absorption spectrum of the hexanucleotide looks like the spectrum in low salt shown in figure 1.

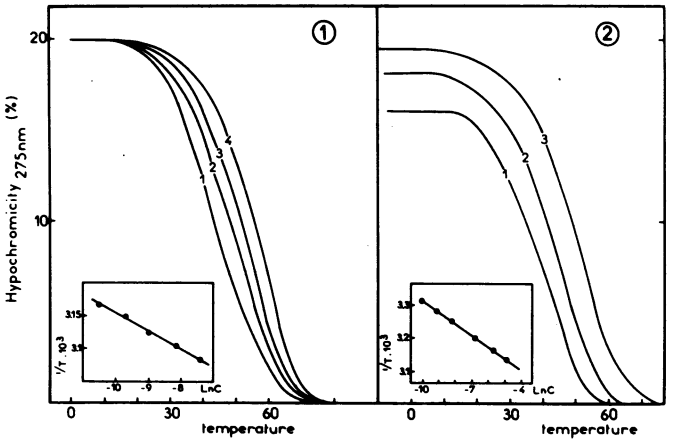

Figure 3 - Ultraviolet changes as a function of temperature. The hypochromicity $H = (A_{80}-A_T)/(A_{80}) \times 100$ where A is the absorbance at a given wavelength, at 80°C and at temperature T versus temperature.
① Solvent 0.2 M $NaClO_4$, 10 mM Tris-HCl pH 7.5 ; $(m^5dC\text{-}dG)_3$ concentration, 1) 5.7 10^{-5} M, 2) 1.15 10^{-4} M, 3) 2.8 10^{-4}, 4) 5.45 10^{-4}.
② Solvent 3 M $NaClO_4$, 10 mM Tris-HCl pH 7.5 ; $(m^5dC\text{-}dG)_3$ concentration, 1) 2.6 10^{-5} M, 2) 2 10^{-4} M, 3) 1.32 10^{-3} M.
Insets ① and ②, Variation of 1/Tm as a function of the logarithm of $(m^5dC\text{-}dG)_3$ concentration.

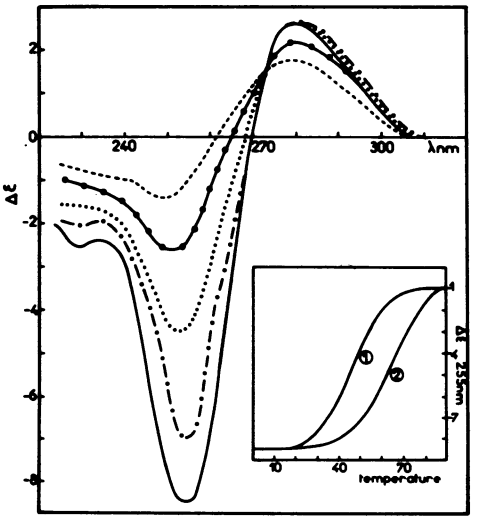

Figure 4 - Circular dichroism. Solvent 0.2 M $NaClO_4$, 10 mM Tris-HCl pH 7.5, temperature 0°C (——), 35°C (—•—), 47°C (···), 57°C (o—o), 80°C (---). $(m^5dC\text{-}dG)_3$ concentration 0.93 10^{-4} M.
Inset : Variation of $\Delta\varepsilon_{255}$ as a function of temperature, $(m^5dC\text{-}dG)_3$ concentration ①, 0.93 10^{-4} M, ②, 2 10^{-3} M.

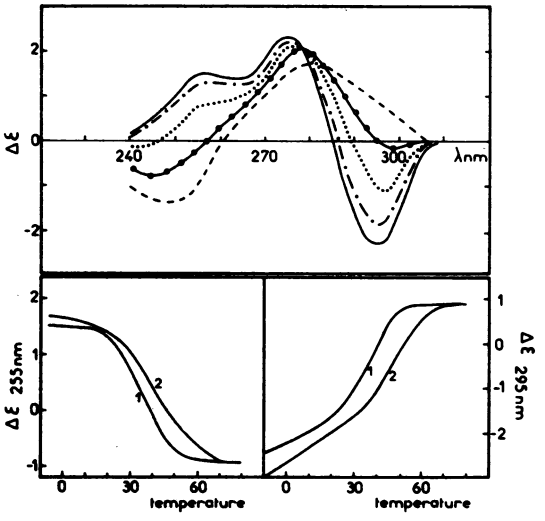

Figure 5 - Circular dichroism. Solvent 3 M $NaClO_4$, 10 mM Tris-HCl pH 7.5, temperature - 9°C (——), 13°C (—•—), 30°C (···), 41°C (—o—), 60°C (---). $(m^5dC\text{-}dG)_3$ concentration 0.95 10^{-4} M.
Insets : Variation of $\Delta\varepsilon_{255}$ (left) and $\Delta\varepsilon_{295}$ (right) as a function of temperature. $(m^5dC\text{-}dG)_3$ concentration 1) 0.95 10^{-4} M, 2) 1.1 10^{-3} M.

Circular Dichroism

The effects of temperature and concentration on the conformation of $(m^5dC\text{-}dG)_3$ have been studied by circular dichroism.

In 0.2 M $NaClO_4$ and at 0°C, the spectrum presents a first positive band centered at 280 nm and then a large negative band centered at 255 nm (figure 4). As the temperature increases, the main changes are a decrease of the band amplitudes with an isoelliptic point at 272 nm. The variations of $\Delta\varepsilon_{255}$ versus temperature is shown in the inset of figure 4. The curve has a sigmoidal shape but the extreme values of $\Delta\varepsilon$ are independent of the hexanucleotide concentration. On the other hand, Tm values are dependent upon the hexanucleotide concentration.

In 3 M $NaClO_4$ and at low temperature, the CD spectrum presents a first negative band centered at 295 nm and a large complex positive band (figure 5). As the temperature is increased, in a large range of temperature, the spectra change gradually with an isodichroic point at 278 nm and then change more abruptly with the disappearance of the isodichroic point. At high temperature, the spectrum presents a positive band and then a negative one and is similar to that oberved at high temperature and in 0.2 M $NaClO_4$ (figure 4).

In the insets of figure 5, are represented the variations of $\Delta\varepsilon_{295}$ and

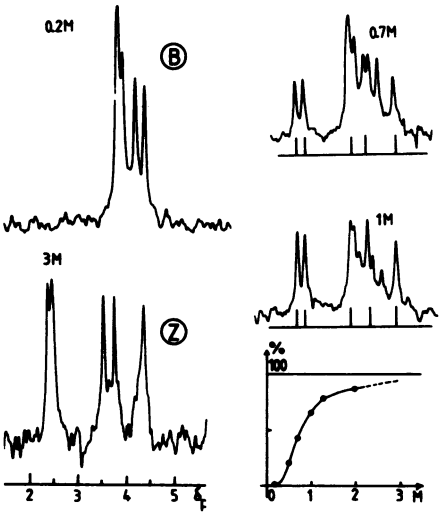

Figure 6 - Proton noise decoupled 36.43 MHz ^{31}P NMR spectra of $(m^5dC\text{-}dG)_3$, c = 6.10^{-3} M, in ^2H_2O:H_2O mixture (2:1), 10 mM Tris-HCl pH 7.5 plus various amounts of $NaClO_4$, temperature 15°C. The spectra at 0.7 M and 1 M $NaClO_4$ are composed of B-form and Z-form spectra (marked by sticks). The percentages of Z-form as a function of salt were evaluated by the relative intensity of low field doublet. Chemical shifts are relative to internal standard trimethylphosphate.

$\Delta\varepsilon_{255}$. The curves (1) are relative to a hexanucleotide concentration 0.95 10^{-4} M. The variation of $\Delta\varepsilon_{295}$ is biphasic. The first transition occurs in a large range of temperature (there is no plateau even at - 8°C) while the second transition is more cooperative. On the other hand, $\Delta\varepsilon_{255}$ is almost constant from - 8°C to 25°C and then increases. The curves (2) are relative to a hexanucleotide concentration 1.4 10^{-3} M. The variation of $\Delta\varepsilon_{295}$ is biphasic. From - 8°C to about 35°C, $\Delta\varepsilon_{295}$ increases linearly and the changes are larger than those observed at low concentrations. The second transition is cooperative. The variation of $\Delta\varepsilon_{255}$ is now biphasic. There is no plateau even at low temperature.

The midpoints of the cooperative transitions at 295 nm correspond roughly to the midpoints of the transition followed by u.v. absorption.

^{31}P Nuclear Magnetic Resonance

The evolution of the proton noise decoupled 36.43 MHz ^{31}P spectra of the hexanucleotide (6 10^{-3} M) at various ionic strength is represented in figure 6. At 15°C and in 0.2 M $NaClO_4$, a partially resolved spectrum spread over a ≅ 0.70 ppm chemical shift range is observed. In 3 M $NaClO_4$, one gets a well-resolved five lines spectrum spread over a ≅ 2.14 ppm chemical shift range.

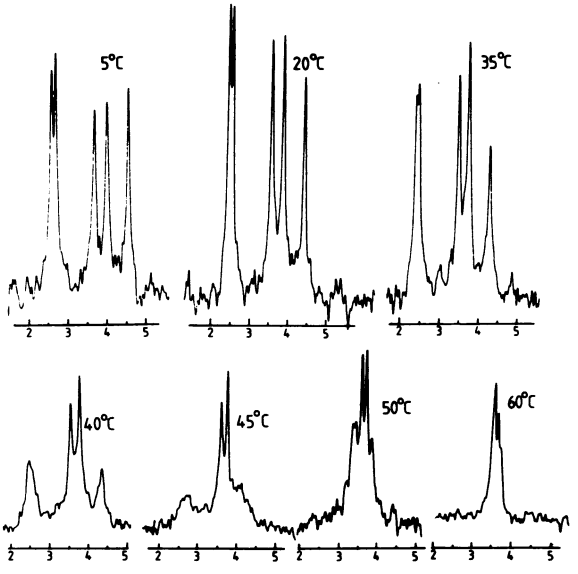

Figure 7 - Proton noise decoupled 36.43 MHz ^{31}P NMR spectra of $(m^5dC\text{-}dG)_3$, c = 6.10^{-3} M, in a ^2H_2O:H_2O mixture (2:1), 10 mM Tris-HCl buffer pH 7.5, 3 M $NaClO_4$ as a function of temperature. The spectra are not normalized. The chemical shifts are relative to internal trimethylphosphate without correction for temperature dependence of the standard.

At intermediate ionic strengths, composite spectra are obtained in which the characteristic spectra obtained at low and high salt concentrations are simply superimposed. Therefore, at 15°C, the equilibrium between the two forms is very slow on the ^{31}P NMR time scale, the exchange frequency being smaller than 5 Hz, the smallest frequency interval between two neighboring resonances of the two forms. In the range 2-5 M $NaClO_4$, one observes a global low field shift of the total spectrum and a slight decrease of the resolution (results not shown).

The temperature dependence of ^{31}P NMR spectra of the hexanucleotide in high salt concentration is reported in figure 7. Between 5 and 35°C, there are continuous changes in the spectra, 1) all the five lines (a, b, c, d, e) are nearly equally downfield shifted (≅ 0.1 ppm), 2) the intensity of the (d, e) doublet decreases whereas that of (b) increases. At temperature higher than 35°C, (a) and (d, e) lines are first broadened and then shifted towards the central part of the spectrum. At 50°C (temperature close to Tm as calculated from the variation of 1/Tm versus Ln c given in figure 3), the rate of conformation exchange depends strongly on temperature as indicated by the appea-

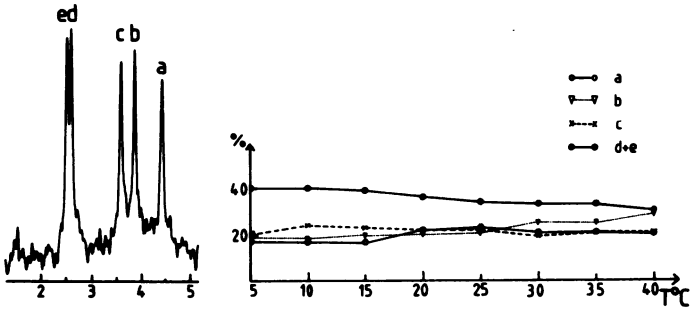

Figure 8 - Changes in the relative intensities of 36.43 MHz ^{31}P NMR spectra of $(m^5dC\text{-}dG)_3$. Same conditions as in figure 7. In addition inverse gated decoupling method was used to suppress nOe. The repetition time was 5s and the accuracy on integral values was ≅ 10 %.

rance of narrow peaks on both sides of the central doublet.

In order to quantify the variations of the spectrum as a function of temperature in the range 5-40°C, we have compared the integrated intensities of the five (a-e) peaks (the nOe was suppressed by using the inverse gated decoupling method). At 5°C, the five peaks have the same intensity, each of them corresponding to one phosphorus. At higher temperatures, the decrease of (d, e) intensity is compensated by an increase of (b) peak intensity. Therefore, there is a conformational change involving both (d) and (e) phosphate groups. A new form appears, spectrum of which should be superimposed to a, b, c lines of the starting high salt form. The equilibrium between these two conformations is slow on ^{31}P NMR time scale, the exchange frequency being smaller than 50 Hz, which is the (d, e)- b frequency interval. At 40°C, their relative proportions are about 75:25.

The effects of temperature were examined at lower hexanucleotide concentration ($2.5\ 10^{-3}$ M). The conformational changes in the range 5-40°C are more complex. The relative intensity of (d, e) doublet decreases as previously, indicating a smaller percentage of the high salt form. Simultaneously, the intensity of (a) singlet slightly decreases whereas that of (c) peak increases (results not shown). These experiments show that the conformation changes are at least partly dependent upon concentration.

DISCUSSION

The conformation of the hexanucleoside pentaphosphate $d(m^5CpGpm^5CpGpm^5CpG)$ depends upon concentration of the oligonucleotide, salt concentration and temperature.

In 0.2 M $NaClO_4$, an ordered structure is formed at low temperature and a disorder one at high temperature, the transition between the two states being cooperative as a function of temperature. This oligonucleotide behaves as several other self-complementary oligonucleotides and in particular as oligo(dG-dC) (19). It can be stated that at low temperature a double helix is formed which belongs to the B-family as judged by determination of molecular weight and by circular dichroism. This double helix dissociates into single strands as the temperature is increased. The enthalpy change $\Delta H°$ for the melting of the double helix is 60 Kcal/mole. It has been found a $\Delta H°$ equal to 50.9 Kcal/mole and 57.4 Kcal/mole for the melting of $(dG\text{-}dC)_3.(dG\text{-}dC)_3$ (19,40).

^{31}P NMR experiments show some differences between this hexanucleotide and $(dC\text{-}dG)_3$ (22). There is large spreading ($\cong$ 0.7 ppm) of the resonance as compared to that of $(dC\text{-}dG)_3$ hexamer duplex ($\cong$ 0.3 ppm). Methylation of cytosine residues modifies the conformational parameters of the phosphodiester backbone and the micro-environment which determine ^{31}P chemical shifts (23).

At low temperature, an increase of salt concentration induces conformational changes. As judged by u.v. absorption and ^{31}P NMR, the transition is complete in 3 M $NaClO_4$ (midpoint of the transition is at about 1.2 M $NaClO_4$). From the sedimentation equilibrium and the spectroscopic data reported here and by comparison with the literature results on oligo(dC-dG), poly(dG-dC). poly(dG-dC) and the corresponding methylated derivatives (1-3, 13-15,24,25), it can be assumed that the ordered form of the hexanucleotide observed at low temperature is a double helix which belongs to the Z-family. At high temperature, the helix dissociates into single strands.

Absorption and circular dichroism experiments reveal a simple behavior for the B or B-like form of $(m^5dC\text{-}dG)_3$. There is no large conformational change before the cooperative melting to single strands and there is no evidence for aggregation. H_{275} and $\Delta\varepsilon_{255}$ do not depend upon concentration (up to 10^{-3} M) and the plateaux are well-defined at low temperature.

The three techniques used in this work show a more complex behavior for the Z-form of $(m^5dC\text{-}dG)_3$. For example, the variation of $\Delta\varepsilon_{295}$ is biphasic and the shape of the curve depends upon hexanucleotide concentration (figure 5).

At low hexanucleotide concentration (at least up to 10^{-4} M), equilibrium ultracentrifuge experiments clearly prove that there is no aggregation. We find the expected molecular weight for an hexamer duplex in 0.2 and 3 M $NaClO_4$ ($\bar{v}$ = 0.64 ml g^{-1} as determined by the method of Edelstein and Schachman, 18). In the same range of concentration, large variations are observed in the CD spectra as the temperature is raised from - 8° to about 30°C, $\Delta\varepsilon_{295}$ changing from

- 2.4 to - 1.4 M^{-1} cm^{-1}. Assuming the rotational strength independent of temperature, such variations reflects an intramolecular change (this change is neither detected at 255 nm in the CD spectra nor at 275 nm in the u.v. spectra). From u.v. spectra, it can be excluded that this transconformation corresponds to a transition towards the B-form as characterized in 0.2 M $NaClO_4$. Such a transition would produce a decrease of the u.v. absorption near 285 nm (see figure 2). It has been carefully checked that this does not occur as the temperature is increased. Thus, one can assume that the decrease of the negative band centered at 295 nm in the C.D. spectrum does not correspond to a Z-form → B-form transition. It can be pointed out that an increase of temperature stabilizes the Z-form of poly(dG-dC).poly(dG-dC) and of poly(dI-br^{5}dC).poly(dI-br^{5}dC) (27-28).

At high concentrations ($\cong 10^{-3}$ M), some spectroscopic parameters are sensitive to concentration effects which can be attributed to aggregation. Aggregation of oligonucleotides has been already reported (26). The structure of aggregates has not yet been resolved. Nevertheless, as discussed below, ^{31}P NMR suggests that end-to-end aggregates could be formed due to the interactions between the terminal base pairs as it occurs in the $(m^5dC\text{-}dG)_3$ and $(dC\text{-}dG)_3$ crystals (1,14).

At high concentration, the slope of the curve $\Delta\varepsilon_{295}$ versus temperature is increased and a slight change of $\Delta\varepsilon_{255}$ is now detected. The hypochromicity is slightly dependent upon hexanucleotide concentration but the experimental accuracy cannot establish unambiguously a variation of u.v. absorbance as a function of temperature. It is important to point out that the solutions were limpid and no light-scattering was detected above 330 nm. In any case, before the melting of the double helix, one has to consider on one hand an aggregation process which depends on concentration and temperature on the other hand a conformational change which depends on temperature.

One can now examine how ^{31}P NMR reflects the properties of $(m^5dC\text{-}dG)_3$ especially in Z-form. Univocal assignments of individual ^{31}P resonances require specific labeling or combined ^{31}P and ^1H NMR experiments in which a sequential assignment of ^1H spectrum has been first done (29). In the present study, it seems reasonable to assign the low field (d, e) doublet to dG2pm^{5}dC3 and dG4pM^{5}dC5 phosphate groups of the hexamer duplex in the Z-form. Indeed, for poly(dG-dC).poly(dG-dC) in Z conformation, the low field ^{31}P peak characterizing this conformation has been univocally assigned to dCpdC phosphate groups (30).

On the other hand, it seems premature to correlate chemical shifts with

conformations of the phosphodiester bonds and a well-defined conformation of the helix. Gorenstein *et al.* have proposed that a (g, g) → (g, t) transition induces a low field shift of ^{31}P resonance (31). This could explain the appearance of the (d, e) doublet in the spectrum of the hexanucleotide in the Z-form, two dGpdC phosphate groups changing their conformations form (g, g) to (g, t). The existence of a (t, g^-) conformation in B-DNA fibers has been recently proposed by several authors from X-ray data (32-34). If such a conformation exists in solution for oligonucleotides, the assumption of Gorestein would not be verified. Moreover, recent theoretical considerations (Giessner-Prettre, personnal communication) suggest that ^{31}P chemical shifts depend also on the O - C torsion angles which makes rather difficult to correlate chemical shifts changes and a precise conformational change.

At room temperature, in aqueous solution, there are important internal motions in a B-helix involving the backbone, the sugar puckering and the base orientations (35,36). Such motions should also exist in a Z helix though they can differ in nature, frequency and amplitude (27,28,37,38). Thus, one cannot assign the low temperature ^{31}P NMR spectrum to a Z_I, Z_{II} or Z' conformations observed in crystals (1-4,14). The conformation existing in solution must be an average conformation resulting from fast exchanges (on NMR time scale) between these conformations and possible others, all belonging to the Z-family

The existence of fast internal motions has been proposed to explain the small dependence of ^{31}P relaxations and nOe's as a function of DNA length (35, 39). An important consequence is that for an hexanucleotide linewidths should be hardly sensitive to aggregation, especially for end-to-end aggregates.

^{31}P NMR experiments have been carried out at hexanucleotide concentrations $2.5\ 10^{-3}$ and $6\ 10^{-3}$ M and aggregation of the duplexes occurs according to the u.v. and C.D. data. At the lower concentration changes in the relative intensities of (a) and (c) peaks as a function of temperature (in the range well below Tm) could indicate some end effects which are detected because of a small degree of aggregation. In parallel there is an intramolecular change. The amount of Z-form decreases as that of a new conformation increases.

At higher concentration, because of larger degree of aggregation end effects are less apparent and the transconformation from the Z-form to the new conformation is more clearly detected especially in 25-40°C range. In spite of these concentration effects, there is a qualitative agreement in the evolution of the Z-form versus temperature in the premelting range as detected by u.v., C.D. and NMR.

In the Z-form, the stereochemical unit is a dinucleotide (1-4). As the

conformational change from the Z-form to the new conformation involves two dGpdC phosphate groups, it seems likely that the two terminal dinucleotides dC1pdG2 and dC5pdG6 are also modified. Several changes can occur as the temperature is raised, i.e., fraying which is a fast kinetic process, premelting of the ends, conformational changes.

On the ^{31}P NMR time scale, only a conformational change is detected which has an effect on the nucleotide backbone and the frequency of which is relatively small. In agreement with u.v. absorption, one can exclude a Z-form → B-form transition, the ^{31}P NMR spectrum of the B-form being not detected. The ^{31}P NMR spectrum of this new conformation is also different from that of single strand hexanucleotide. All these results suggest that this new conformation corresponds to a distortion of the Z-form double helix leading to a more regular form in which the internal phosphate groups have a more similar environment.

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REFERENCES

1. Wang, A.H.J., Quigley, G.S., Kolpak, F.J., Crawford, J.L., van Boom, J.H., van der Marel, G. and Rich, A. (1979) Nature (London) 282-680-686.
2. Wang, A.H.J., Quigley, G.S., Kolpak, F.J., van der Marel, G., van Boom, J.H. and Rich, A. (1981) Science 211, 171-176.
3. Drew, H., Takano, T., Tanaka, S., Itakura, K. and Dickerson, R.E. (1980) Nature (London) 286, 567-573.
4. Rich, A. (1983) in Structure, Dynamics, Interactions and Evolution of Biological Macromolecules, C. Hélène, ed., D. Reidel Publishing Company, Dordrecht, Holland, pp. 3-21.
5. Lafer, E.M., Möller, A., Nordheim, A., Stollar, B.D. and Rich, A. (1981) Proc. Natl. Acad. Sci. USA 78, 3546-3550.
6. Malfoy, B. and Leng, M. (1981) FEBS Letters 132, 45-48.
7. Nordheim, A., Pardue, M.L., Lafer, E.M., Möller, A., Stollar, D.B. and Rich, A. (1981) Nature 294, 417-422.
8. Leng, M., Hartmann, B., Malfoy, B., Pilet, J., Ramstein, J. and Sage, E. (1982) 47th Symposium on Quantitative Biology, Cold Spring Harbor, in press.
9. Lemeunier, F., Derbin, C., Malfoy, B., Leng, M. and Taillandier, E. (1982) Exp. Cell. Res. 141, 508-513.
10. Lipps, H.J., Nordheim, A., Lafer, E.M., Ammermann, D., Stollar, D.B. and Rich, A. (1983) Cell 32, 435-441.
11. Morgenegg, G., Celio, M.R., Malfoy, B., Leng, M. and Kuenzle, C.C. (1983) Nature (London) in press.
12. Leng, M. (1983) in Structure, Dynamics, Interactions and Evolution of Biological Macromolecules, C. Hélène, ed., D. Reidel Publishing Company, Dordrecht, Holland, pp 45-56.

13. Behe, M. and Felsenfeld, G. (1981) Proc. Natl. Acad. Sic. USA 78, 1619-1623.
14. Fujii, S., Wang, A.H.J., vand der Marel, G., van Boom, J.H. and Rich, A. (1982) Nucleic Acids Res. 10, 7879-7892.
15. Thamann, T.J., Lord, R.C., Wang, A.H.J. and Rich, A. (1981) Nucleic Acids Res. 9, 5443-5457.
16. Quadrifoglio, F., Manzini, G., Vasser, M., Dinkelspiel, K. and Crea, R. (1981) Nucleic Acids Res. 9, 2195-2206.
17a Thuong, N.T., Chassignol, M., Lancelot, G., Mayer, R., Hartmann, B., Leng, M. and Hélène, C. (1981) Biochimie 63, 775-784.
17b Thuong, N.T., Barbier, C. and Asseline, U. (1983) Phosphorus and Sulfur, 14, 357-365.
18. Edelstein, S.J. and Schachman, H.K. (1967) J. Biol. Chem. 242, 306-311.
19. Pohl, F.M. (1974) Eur. J. Biochem. 42, 495-504.
20. Applequist, J. and Damle, B. (1965) J. Am. Chem. Soc. 87, 1450-1458.
21. Eigen, M. and Porschke, D. (1970) J. Mol. Biol. 53, 123-141.
22. Patel, D.J. (1976) Biopolymers 15, 533-558.
23. Ribas Prado, F., Giessner-Prettre, C., Pullman, B. and Daubey, J.P. (1979) J. Am. Chem. Soc. 101, 1737-1742.
24. Pohl, F.M. and Jovin, T.M. (1972) J. Mol. Biol. 67, 375-396.
25. Behe, M., Zimmerman, S. and Felsenfeld, G. (1982) Nature 293, 233-235.
26. Nelson, J.W., Martin, F.H. and Tinoco, I. (1981) J. Mol. Biol. 20, 2509-2531.
27. Patel, D.J., Kozlowski, S.A., Nordheim, A. and Rich, A. (1982) Proc. Natl. Acad. Sci. USA 79, 1413-1417.
28. Hartmann, B., Pilet, J., Ptak, M., Ramstein, J., Malfoy, B. and Leng, M. (1982) Nucleic Acids Res. 10, 3261-3277.
29. Cheng, D.M., Kan, L.S., Miller, D.S., Leutzinger, E.E. and Ts'O, P.O.P. (1982) Biopolymers 697-701.
30. Jovin, T.M., van de Sande, J.H., Zarling, D.A., Ardnt-Jovin, D.J., Eckstein, F., Fuldner, H.H., Greider, C., Grieger, I., Hamori, E., Kalisch, B., Mc Intosh, L.P. and Robert-Nicoud, M. (1982) 47th Symposium on Quantitative Biology, Cold Spring Harbor, in press.
31. Gorenstein, D.G., Luxon, B.A., Goldfield, E.M., Lai, K. and Vegemij, D. (1982) Biochemistry 21, 580-589.
32. Arnott, S., Chandrasekaran, R., Birdsall, D.L., Leslie, A.G.W. and Ratliff, R.L. (1980) Nature 283, 743-745.
33. Gupta, G., Bansal, M. and Sasisekharan, V. (1980) Proc. Natl. Acad. Sci. USA 77, 6486-6490.
34. Ramaswamy, N., Bansal, M. Gupta, G. and Sasisekharan, V. (1982) Proc. Natl. Acad. Sci. USA 79, 6109-6113.
35. Hogan, M.E. and Jardetzky, O. (1980) Biochemistry 79, 3460-3468.
36. Keepers, J.W. and James, T.L. (1982) J. Am. Chem. Soc. 104, 929-939.
37. Ramstein, J. and Leng, M. (1980) Nature (London) 288, 413-414.
38. Pilet, J. and Leng, M. (1982) Proc. Natl. Acad. Sci. USA 79, 26-30.
39. Hogan, M.E. and Jardetzky, O. (1979) Proc. Natl. Acad. Sci. USA 76, 6341-6345.
40. De Prisco Albergo, D., Marky, L.A., Breslauer, K.J. and Turner, D.H. (1981) Biochemistry 20, 1409-1413.
41. Neimark, J. (1975) Thesis University Louis Pasteur, Strasbourg.