NMR Relaxation and the Structure of a Synthetic DNA poly(dA-dT) . poly(dA-dT)

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Abstract. ^{1}H—^{1}H and ^{31}P—^{1}H nuclear Overhauser effects and ^{31}P NMR spin-lattice relaxation times were measured for a synthetic DNA poly(dA-dT) . poly(dA-dT) in a low-salt aqueous solution. The results have shown that all bases in the double helix are anti-orientated with respect to deoxyribose residues and that the sugar-phosphate backbone has an alternating architecture.

Key words: NMR relaxation — NOE — T_1 — DNA — poly(dA-dT) . poly(dA-dT) — Alternating structure — Glycosidic torsion angles

Introduction

The combination of single crystal X-ray diffraction analysis (Dickerson et al. 1982) and NMR studies in solution (Patel et al. 1982b) has explicitly established that DNA conformation is dictated by base sequence. Though sufficient data have been accumulated to justify this generalization the situation is far from providing a vocabulary to translate base sequence into DNA conformation. As one example of an empty space in this vocabulary serves the alternating sequence of adenylyl (dA) and thymidylyl (dT) residues. There have been attempts to crystalize d(ATATATAT), d(ATATAT) and d(ATAT) oligomers but with the exception of the latter compound the crystals were unstable and unsuitable for X-ray diffraction analysis (Shakked et al. 1981). The tetranucleotide d(ATAT) crystallized properly but its crystal structure was rather surprising. It did not form the self-paired duplex of four base pairs as expected for its self-complementary sequence (Viswamitra et al. 1978). Instead, the first two bases of the tetramer paired in the Watson-Crick manner with complementary bases from another tetramer, but then the backbone swung sharply away from the helical direction, allowing the third and fourth bases to pair with a third tetramer. The sharp swing completely cancelled stacking interactions between the bases in the central dT-dA step of the d(ATAT) tetramer

(Viswamitra et al. 1978). The peculiar structural properties of the tetramer organization in crystal, though they cannot unambiguously be extrapolated to the double helix, inspired the proposal of the alternating B conformation of poly(dA-dT) . poly(dA-dT) (Klug et al. 1979).

It is now also possible to study DNA structure in solution at atomic resolution. Such studies employ NMR relaxation techniques that permit determination of interproton distances in short DNA fragments (for a review, see Kearns 1984). Informative is the nuclear Overhauser effect (NOE, for a review, see Noggle and Schirmer 1971) whose measurements allow specification of protons close in space (Patel et al. 1983). Let us consider two ^{1}H NMR resonances designated A and B that belong to protons separated by, say, less than 0.3 nm. Then irradiation of B brings about NOE — a change in the intensity of A, and vice versa. No NOE is, however, observed between resonances that are separated by 0.5 nm or more since NOE is extremely sensitive to the distance between the irradiated and observed protons (NOE decreases with the sixth power of this distance). Similar information about DNA solution structure is also contained in the spin-lattice relaxation times, T_1, of its NMR resonances (Feigon et al. 1983).

Here we report results of ^{1}H-^{1}H and ^{31}P-^{1}H NOE and ^{31}P NMR T_1 measurements on poly(dA-dT) . poly(dA-dT) in low-salt, aqueous solution and consider their structural implications in the framework of the relevant literature data.

Materials and Methods

Poly(dA-dT) . poly(dA-dT) was a product of PL Biochemicals. As NMR spectra with a better resolution and from a substantial less number of scans can be obtained with short than long DNA fragments we sonicated the polynucleotide as described previously (Vorlíčková et al. 1983). The sonicated sample was characterized by circular dichroism and thermal denaturation curve. No significant deviations from the original highly polymerized material were found. Polyacrylamide gel electrophoresis showed that the length distribution of the sonicated material was relatively narrow. Its band maximum was centered around 100 base pairs if an Alu I digest of pBR 322 plasmid DNA was used as the length marker (Sutcliffe 1978).

^{1}H NMR spectra at different temperatures were obtained with 2 mg of the sonicated poly(dA-dT) . poly(dA-dT) dissolved in 0.4 ml of 0.1 mol/l sodium phosphate, 0.1 mmol/l EDTA, D_2O, pH 7. The sample was lyophilized, dissolved in 99.9% D_2O, repeatedly lyophilized, and finally dissolved in ultrapure 99.996% D_2O (Stohler Isotope Chemicals) to minimize the residual water resonance. Chemical shifts were referenced with respect to the internal standard, 2, 2-dimethyl-2-silapentane-5-sulfonate (DSS). Interproton Overhauser effects were measured with 1 mg of the sonicated poly(dA-dT) . poly(dA-dT) dissolved in 0.13 ml of the phosphate buffer and transferred to a special Wilmad 508 cp NMR microcell. Preparatory procedures were identical as above except that the sample was once more lyophilized from 99.9% D_2O. ^{31}P NMR measurements were performed with 3 mg of the sonicated poly(dA-dT) . poly(dA-dT) dissolved in 0.4 ml of 10 mmol/l Tris-HCl (50% D_2O), 100 mmol/l NaCl, 1 mmol/l EDTA, pH 7.5, and 10 mmol/l trimethylphosphate was used as the internal NMR standard. All the ^{31}P NMR measurements were carried out at 30 °C. Prior to NMR relaxation measurements, the polynucleotide samples were passed through a Chelex-100 column and

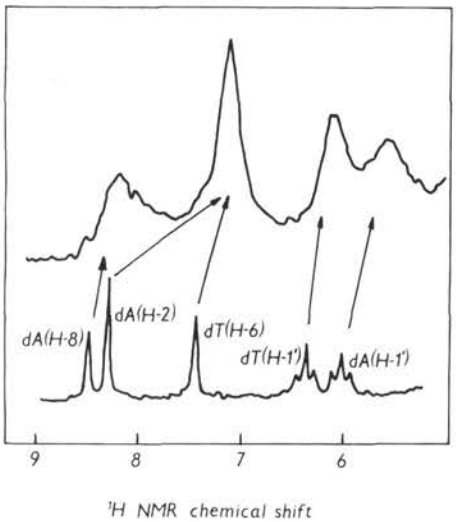

Fig. 1. 80 MHz ^{1}H NMR spectra of sonicated poly(dA-dT) in the duplex (40 °C, *top*) and single-stranded (80 °C, *bottom*) conformational state.

bubbled thoroughly with dry nitrogen to remove paramagnetic impurities and oxygen.Then the NMR cells were sealed with Parafilm.

An adapted Bruker WP 80 NMR spectrometer with an Oxford cryomagnet (magnetic field of 4.63 T) was used for ^{31}P NMR measurements at 80 MHz. Spectra were recorded with a deuterium lock under proton broadband decoupling. Typically 1000 scans were sufficient to obtain a good signal-to-noise ratio with 8 K data points, sweep width 1875 Hz, delay time 2.5 s, and a flip angle 65° (14 microsec). Spin-lattice relaxation times, T_1, and nuclear Overhauser effects, NOE, were determined by fast inversion recovery (Sass and Ziessow 1977) and gated decoupling technique (Freeman and Hill 1971), respectively. Fast inversion recovery experiments (pulse sequence T_D-π-τ-$\pi/2$-AQT) were carried out with the following parameters: pulse length $\pi = 38$ microsec, time delay $T_D = 3.6$ s . T_1 relaxation times were estimated using the three parameter nonlinear least square fit of the observed data to the exponential function (Sass and Ziessow 1977). ^{31}P-^{1}H NOE values were taken from the difference spectra obtained with the broadband decoupling switched on and off during the equilibration period prior to the $\pi/2$ observation pulse. The length of this period was adjusted to 21.1 s (8 times the T_1 measured). ^{1}H-^{1}H NOEs were estimated from the relative change of resonance amplitudes. However, selective homonuclear decoupling with a frequency adjusted to signals of interest was employed. The spectra were obtained with a 7.1 s equilibration period.

Results and Discussion

Figure 1 shows an aromatic base and anomeric sugar part of the ^{1}H NMR spectrum of poly(dA-dT) . poly(dA-dT) at low and high temperature. In the latter case the polynucleotide is predominantly single-stranded, its chains are coiled, mobile and thus the NMR resonances are relatively very narrow. Even the triplets of the

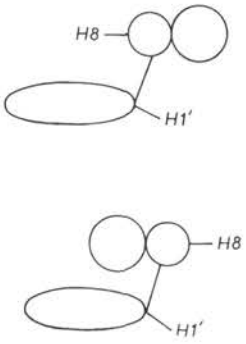

Fig. 2. Schematic drawing of the anti (*top*) and syn (*bottom*) conformation around the glycosidic bond in a purine residue.

anomeric H1' protons are clearly resolved due to J coupling to the H2′ and H2″ deoxyribose protons. The spectrum makes possible the signal assignment according to the published data (Patel 1978) and it also shows that the sample contains no contaminations. When the temperature was slowly decreased the resonances shifted upfield and became substantially wider. This phenomenon is known to accompany the formation of the polynucleotide duplex (Patel 1978). The duplex is stiffer and less mobile than the coil, which turns the resonances wider. In addition, bases are placed into the interior of the double helix, where they are shielded by other bases. This is the reason for the base protons to exhibit upfield shifts (Fig. 1). The shift is most pronounced with the dA(H2) proton that is located near the helix centre. Its large shift brings about that the dA(H2) and dT(H6) resonances overlap in the duplex state.

Probably the most significant parameter that decides about the global double helix architecture is the glycosidic torsion angle (Rao and Sasisekharan 1983). The glycosidic torsions of nucleotide residues in DNA mostly occupy the anti region. Then the parts of bases capable of forming Watson-Crick hydrogen bonds are turned away from the sugar rings (Fig. 2). In the anti orientation, no interproton Overhauser effect is expected between purine (H8) or pyrimidine (H6) and the respective H1′ sugar protons since they are 0.38 nm apart (Patel et al. 1982a). On the other hand, in the syn orientation (which appears in the left-handed Z-DNA helix at the purine residues (Wang et al. 1979) and overall in the right-handed Hopkins model of DNA (Hopkins 1981)) when the bases are located over the sugar rings the purine (H8) protons are 0.22 nm away from H1′. Then the Overhauser effect is anticipated and was actually observed with the Z form (Patel et al. 1982a).

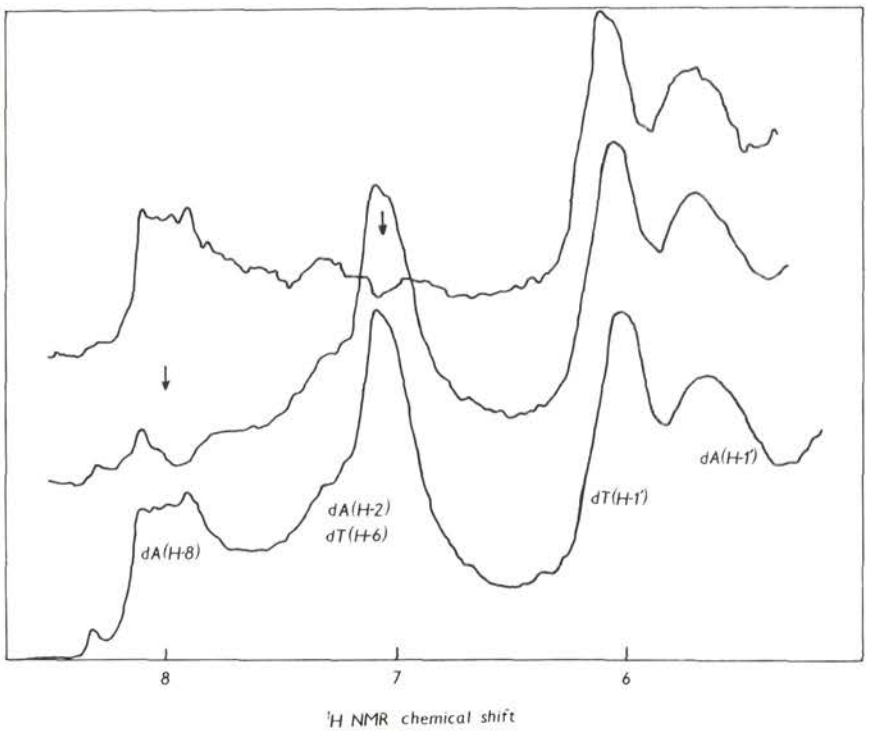

Fig. 3. ^{1}H-^{1}H NOE experiments on sonicated poly(dA-dT) . poly(dA-dT). Arrows indicate the irradiated resonances: overlapping dA(H2) and dT(H6) — *top*; dA(H8) — middle; control spectrum with no irradiation — *bottom*. Temperature 57 °C.

The results of our ^{1}H-^{1}H NOE experiments on poly(dA-dT) . poly(dA-dT) in low-salt solution are shown in Figure 3. We selectively irradiated dA(H8) or dT(H6) + dA(H2) protons and followed the amplitudes of the dA(H1′) and dT(H1′) resonances. The amplitudes did not change in either case, which indicates that both dA and dT residues are anti in poly(dA-dT) . poly(dA-dT). The polynucleotide double helix thus can neither be the Z form nor the Hopkins structure. A small decrease in the amplitude of dT(H6) + dA(H2) was observed when dA(H8) was irradiated. This effect is not of a conformational origin. It can be a consequence, as suggested by a referee, of insuficient selectivity in the dA(H8) irradiation, which would result in partial saturation and thus an amplitude decrease of the dA(H2) + dT(H6) resonance.

Much about DNA architecture can also be disclosed by studying the geometry of the phosphodiester bonds, the most flexible part of the DNA molecule (Yathindra and Sundaralingam 1976). Their geometry is conveniently reflected in

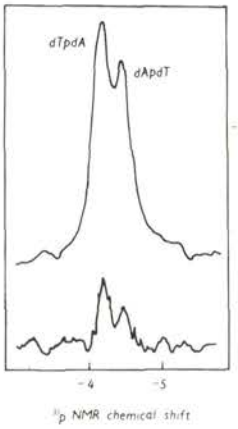

Fig. 4. 80 MHz ^{31}P NMR spectrum of sonicated poly(dA-dT) . poly(dA-dT) with ^{31}P-^{1}H NOE (*top*). Difference spectrum with and without NOE (*bottom*). The designations dTpdA and dApdT refer to phosphorus resonances in the dT-dA and dA-dT steps, respectively. For details, see text.

the ^{31}P NMR spectra (Gorenstein 1981). As originally observed by Shindo et al. (1979), the ^{31}P NMR spectrum of poly(dA-dT) . poly(dA-dT) contains two partially resolved resonances of equal intensity (Fig. 4). It was surprising since all natural DNAs and other polynucleotides examined so far have given only a single resonance. The authors have excluded several possible origins of the resonance splitting and have finally suggested that the separation reflected a different geometry of the phosphodiester bonds at the dApdT and dTpdA phosphates. Some workers, however, do not take this explanation into account and claim that the splitting is just a sequence, i. e. chemical and not a conformational effect (Gupta et al. 1983). We therefore decided to measure relaxation parameters of the two ^{31}P NMR resonances of poly(dA-dT) . poly(dA-dT) that are sensitive to the lengths of the P-H vectors, where H are deoxyribose protons close in space to phosphorus P. If the geometries of phosphodiester bonds in the dApdT and dTpdA steps were actually different then their ^{31}P NMR resonances should have different ^{31}P-^{1}H NOEs and T_1s.

The left peak in the ^{31}P NMR spectrum of poly(dA-dT) . poly(dA-dT) (Fig. 4) corresponds to dTpdA phosphates and the other peak to dApdT phosphates. The assignment followed from a phosphorothioate modification of the polynucleotide phosphates (Eckstein and Jovin 1983). ^{31}P NMR measurements are usually performed with the broadband proton decoupling which increases the sensitivity of ^{31}P NMR measurements due to the positive ^{31}P-^{1}H NOE and the collapse of multiplet resonances caused by the ^{31}P-^{1}H scalar interactions. In the case of

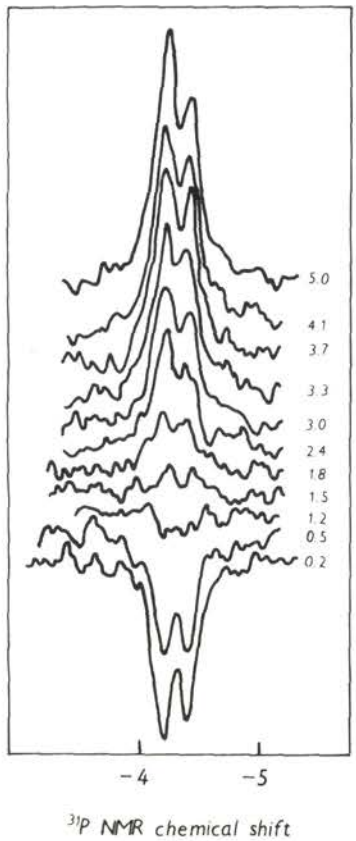

Fig. 5. Measurement of the ^{31}P NMR T_1s of sonicated poly(dA-dT) . poly(dA-dT). The numbers indicate relaxation delays τ in seconds.

polydeoxynucleotides, deoxyribose H3′, H5′ and probably H2′ protons may contribute to the effect. The spectrum of poly(dA-dT) . poly(dA-dT) with NOE is shown at the top of Figure 4 and at the bottom there is a difference between the spectra obtained in the presence and absence of NOE. It is immediately clear from the difference spectrum that the dTpdA resonance has a higher NOE and thus the geometries of the dApdT and dTpdA phosphohodiesters cannot be identical. Numerical values are: NOE (dTpdA) = 28%, NOE(dApdT) = 15%.

We also measured spin-lattice relaxation times, T_1, of the ^{31}P NMR resonances of poly(dA-dT) . poly(dA-dT). Two relaxation mechanisms contribute to the magnitude of this quantity (for a review, see James 1984). The first one is a consequence of the chemical shift anisotropy. Its contribution depends on the magnetic field. Under the conditions of our experiments, it accounts for 35% of the

^{31}P NMR relaxation of poly(dA-dT) . poly (dA-dT) (James 1984). The other relaxation mechanism is dipolar. Its efficiency depends on the P-H internuclear distances that may vary with the conformation of the DNA sugar-phosphate backbone. Unlike chemical shift anisotropy, the contribution of the dipolar relaxation to T_1 provides useful information about the disposition of deoxyribose protons with respect to phosphate groups. Base protons are too remote to have an influence.

Spectra used to derive T_1s of the ^{31}P NMR resonances of poly(dA-dT) . poly(dA-dT) are shown in Figure 5. Here we can see that the resonance amplitudes depend upon the variable relaxation delay, τ, between the π and $\pi/2$ pulses. We found that T_1s were not identical for the two ^{31}P NMR resonances of poly(dA-dT) . poly(dA-dT) (T_1(dTpdA) = 2.5 s, T_1(dApdT) = 2.1 s). This finding indicates that deoxyribose protons are not identically located in the dApdT and dTpdA steps of the duplex with respect to phosphate groups; this along with the results of the ^{31}P-^{1}H NOE measurements suggests that the backbone of poly(dA-dT) . poly(dA-dT) is not smooth and regular as it is the case in the classical Watson-Crick model of DNA. Instead, it periodically alternates with a repeat of two nucleotide residues.

Conclusions

We could demonstrate two things. First, all residues are in an anti orientation around the glycosidic bond in poly(dA-dT) . poly(dA-dT) at low-salt, thus excluding the possibility that it assumes a left-handed Z-DNA or a Hopkins' structure. Nonetheless, many alternatives remain as to what structure the polynucleotide actually adopts. The other main experimental result of this study following from ^{31}P NMR relaxation measurements implies that the synthetic DNA structure is alternating rather than regular. Our data thus contradict suggestions that the synthetic DNA is a uniform right-handed (Mellema et al. 1983) or a uniform left-handed (Gupta et al. 1983) B helix. Though it is not possible to discriminate between the right-handed and the left-handed sense of the double helix winding at the moment, several pieces of indirect evidence are in support to the former alternative: (i) Chiroptical properties of poly(dA-dT) . poly(dA-dT) and of the known right-handed molecules of DNA are similar. On the other hand, all known left-handed molecules of DNA have chiroptical properties substantially different (Pohl and Jovin 1972; Ivanov and Minyat 1981; McIntosh et al. 1983). (ii) Recombinant plasmids containing $(dA\text{-}dT)_n$. $(dA\text{-}dT)_n$ fragments have been constructed and analysed by the band shift method (Wang 1979) which is sensitive to relative handedness of the DNA double helix in the parent plasmid and in the inserted sequence. No indication of opposite handedness has, however, been reported (Strauss et al. 1981). (iii) Repressors require right-handed DNA for

binding (Takeda et al. 1983) and, consequently, poly(dA-dT) . poly(dA-dT) is very unlikely left-handed since it binds lac repressor much strongly than calf thymus DNA (Riggs et al. 1972). Taken altogether, it seems that poly(dA-dT) . poly (dA-dT) assumes a right-handed alternating B-DNA conformation though its structural coordinates suggested from theoretical considerations (Klug et al. 1979) may not be correct. They do not, for example, account for the absence of the interproton Overhauser effect between the dT(H6) and dT (H2′, H2″) nuclei (Gupta et al. 1983).

In conclusion, we would like to summarize the experimental data concerning the low-salt structure of poly(dA-dT) . poly(dA-dT) that appear to implicate unambiguous structural properties: (i) Bases are hydrogen bonded in the classical Watson—Crick manner (Gupta et al. 1983). (ii) All glycosidic torsions are anti (Gupta et al. 1983; this work). (iii) Adenine and thymine residues have different sugar puckerings (Shindo 1981). (iv) Phosphodiesters in the dA-dT and dT-dA steps have different geometries (Shindo et al. 1979; this work). (v) The structure of poly(dA-dT) . poly(dA-dT) is alternating, which in addition to the NMR data, also follows from the fact that DNase I cuts the polynucleotide dinucleotide after dinucleotide (Scheffler et al. 1968). (vi) Most likely, the structure is right-handed, as indicated by the data summarized above. Nonetheless, it is far from being conclusively specified and hence will be a subject of future studies. They will not only be interesting from the structural point of view but also because alternating dA-dT regions play significant regulatory roles in DNA functions (Wasylyk et al. 1980).

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Note

We have learnt in the course of the manuscript preparation that other authors (Assa-Munt N. and Kearns D. R. (1984) Biochemistry **23**, 791—796) obtained similar ^{1}H-^{1}H NOE results on poly(dA-dT) . poly(dA-dT).

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