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Accelerated Publications

Hybrid Hexanucleotide Duplex Containing Cyclonucleotides and Deoxynucleotides: The d(TA) Segment Can Adopt a High Anti Left-Handed Double-Helical Structure†

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ABSTRACT: It is known that oligonucleotides containing cyclonucleosides with a high anti (intermediate between anti and syn) glycosidic conformation adopt left-handed, single- and double-helical structures [Uesugi, S., Yano, J., Yano, E., & Ikehara, M. (1977) *J. Am. Chem. Soc. 99*, 2313–2323]. In order to see whether DNA can adopt the high anti left-handed double-helical structure or not, a self-complementary hexanucleotide containing 6,2′-*O*-cyclocytidine (C°), 8,2′-*O*-cycloguanosine (G°), thymidine, and deoxyadenosine, C°G°dTdAC°G°, was synthesized. Imino proton NMR spectra and the results of nuclear Overhauser effect experiments strongly suggest that C°G°dTdAC°G° adopts a left-handed double-helical structure where the deoxynucleoside residues are involved in hydrogen bonding and take a high anti glycosidic conformation. A conformational model of the left-handed duplex was obtained by calculation with energy minimization. Thus it appears that DNA can form a high anti, left-handed double helix under some constrained conditions, which is quite different from that of Z-DNA.

8,2′-*S*-Cycloadenosine (A^s),[1] where the adenine C8 and sugar C2′ are bridged with a sulfur atom, has a fixed glycosidic torsion angle in a high anti region (Prusiner et al., 1973; Tanaka et al., 1979). In the course of studying oligonucleotide derivatives of purine cyclonucleosides, we found that a dinucleoside monophosphate of 8,2′-*S*-cycloadenosine, A^{s}pA^s, takes a stable stacking conformation with a left-handed screw axis (Ikehara et al., 1970; Uesugi et al., 1972). Homooligonucleotides containing A^s adopt a left-handed helical structure (Ikehara & Uesugi, 1972) and form left-handed duplexes with homooligonucleotides containing 6,2′-*O*-cyclouridine (U°), which has the same glycosidic conformation as that of A^s (Uesugi et al., 1976). Studies on ApA analogues containing cyclonucleosides with different torsion angles in an anti range and adenosine reveal that the high anti conformation (χ = 110–120° according to Sundaralingam's definition; Sundaralingam, 1969) is required for most stable left-handed stacks (Uesugi et al., 1977). Studies on pairs of dimer sequence isomers containing different base species (adenine, uracil, and hypoxanthine) confirmed the left-handedness of stacking (Ikehara et al., 1980; Uesugi et al., 1980a,b). These studies also revealed that the order of sequence-dependent stability of stacking (U°pA^s > A^{s}pU°) and the order of sequence-dependent stability of the ethidium complex (A^{s}pU° > U°pA^s) for A^{s}pU° and U°pA^s are reversed with respect to those for ApU and UpA. These phenomena are a reflection of the difference in the modes of stacking in left-handed and right-handed stacks. Energy calculations on the A^{s}pA^s conformation also support the view that the left-handed stack is the most stable one (Fujii & Tomita, 1976). Extension of the dimer structure to a polymer structure gives a regular left-handed helix with no unusual conformational parameters, including those for the phosphodiester bonds (Fujii & Tomita, 1976; Yathindra & Sundaralingam, 1976).

Discovery of the Z-DNA structure (Wang et al., 1979), which adopts quite different left-handed double-helical structure from the high anti one, and subsequent studies on DNA structures in crystals, fibers, and solution revealed that DNA is conformationally very flexible. On the basis of these advances, it was of great interest to see whether DNA can adopt the high anti left-handed double-helical structure or not. For this purpose, we synthesized a self-complementary hexanucleotide containing 6,2′-*O*-cyclocytidine (C°), 8,2′-*O*-cycloguanosine (G°), thymidine, and deoxyadenosine, C°G°dTdAC°G°, in which a d(TA) segment is sandwiched between two C°G° segments (Figure 1). It was expected that the flanking C°G° segments might force the d(TA) segment

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[1] Abbreviations: A^s, 8,2′-anhydro-9-β-D-arabinofuranosyl-8-mercaptoadenine; U°, 6,2′-anhydro-1-β-D-arabinofuranosyl-6-hydroxyuracil; C°, 6,2′-anhydro-1-β-D-arabinofuranosyl-6-hydroxycytosine; G°, 8,2′-anhydro-9-β-D-arabinofuranosyl-8-hydroxyguanine; NOE, nuclear Overhauser effect; DSS, sodium 1-(trimethylsilyl)propane-3-sulfonate; EDTA, ethylenediaminetetraacetic acid.

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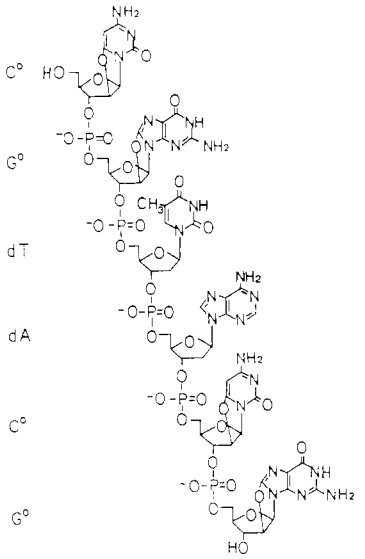

FIGURE 1: Chemical structue of C°G°dTdAC°G°.

to take a high anti left-handed double-helical structure.

Materials and Methods

Synthesis of C°G°dTdAC°G°. G° was synthesized according to the published method (Ikehara & Maruyama, 1975). C° was synthesized by a modified method of Maruyama et al. (1982). C°G°dTdAC°G° was synthesized by a modified phosphotriester method (Ohtsuka et al., 1981). After deprotection, the oligomer was purified by reversed-phase chromatography on a column of alkylated silica gel (C_{18}, Waters) and gave a single peak on analysis by high-performance liquid chromatography on a column of alkylated silica gel.

NMR Measurement. A sample of the sodium salt for NMR measurement was prepared by successive treatment with Dowex 50 (pyridinium form), Dowex 50 (sodium form), and Chelex 100 resins. ^{1}H NMR spectra (500 MHz) were recorded on a JEOL GX500 spectrometer. The chemical shifts were measured downfield from internal 2-methyl-2-propanol, which has been referenced to sodium 1-(trimethylsilyl)-propane-3-sulfonate (DSS). ^{1}H NMR spectra in the imino proton region were measured in H_2O containing 20% D_2O with a time-shared hard 1-1 pulse sequence to suppress the strong H_2O signal (Clore et al., 1983). NOE difference spectra were obtained by subtraction of accumulated off-resonance spectra from the on-resonance spectra. A single-frequency preirradiation pulse was applied for 0.2–0.5 s with an irradiation power level to give about 60% saturation of the irradiated signal.

Conformational Energy Analysis. Conformational analysis of the hybrid hexamer was carried out by using the molecular mechanics calculation package that was developed from the AMBER program (Weiner & Kollman, 1981). Several starting models that adopt the left-handed duplex structure and have good base stacking had been built by using a space-filling model (CPK model) and a computer graphics system. The conformational energy calculations were performed without solvent molecules and with sodium ions as counterion by applying a distance-dependent dielectric constant. We have employed Weiner's force field parameters (Weiner et al., 1984) and have also calculated and defined the additional parameters involved in covalent linkages between the base and sugar moieties using X-ray structures of C° (Yamagata et al., 1979) and 8,2′-*O*-cycloadenosine (Neidle et al., 1979). The structures were refined until the root-mean-square gradient was less than 0.1 kcal/(mol Å).

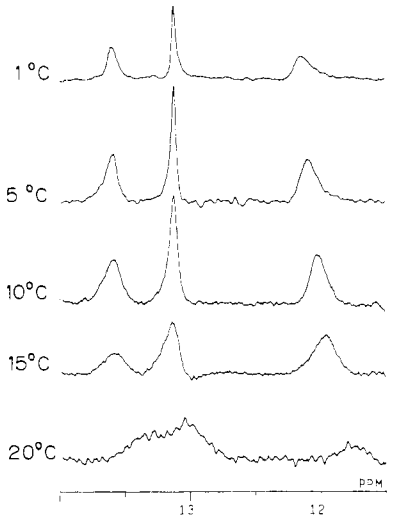

FIGURE 2: Imino proton NMR spectra of C°G°dTdAC°G° (strand concentration, 5.7 mM) in 0.1 M NaCl, 0.01 M sodium phosphate buffer (pH 7.2), and 0.1 mM EDTA containing 20% D_2O at various temperatures (1, 5, 10, 15, and 20 °C).

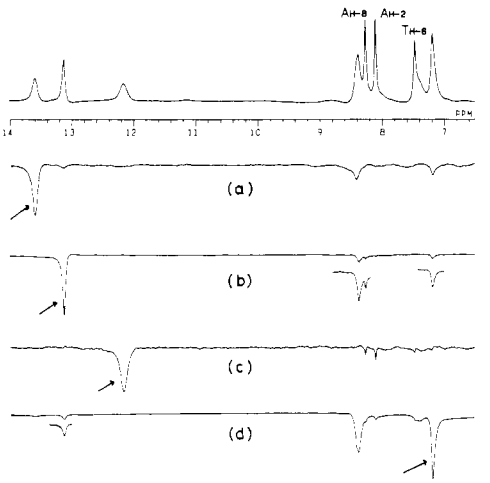

FIGURE 3: Proton NMR spectra (7–14 ppm downfield from DSS) of C°G°dTdAC°G° (strand concentration, 5.7 mM) in 0.1 M NaCl, 0.01 M sodium phosphate buffer (pH 7.2), and 0.1 mM EDTA containing 20% D_2O at 1 °C. A normal spectrum is shown at the top. (a–d) NOE difference spectra obtained by irradiating the signals (indicated with an arrow) at 13.58 (a), 13.11 (b), 12.13 (c), and 7.08 ppm (d).

Results

Imino Proton NMR Spectra. Figure 2 shows the low-field (11–14 ppm downfield from DSS) NMR spectra of C°G°dTdAC°G° in H_2O at 1–20 °C. It is known that the hydrogen-bonded imino proton resonances of guanine, thymine, and uracil residues are observed in this region (Kearns et al., 1971). The spectrum of C°G°dTdAC°G° at 1 °C shows three, clearly resolved signals (at 13.58, 13.11, and 12.13 ppm), which correspond to one thymine and two guanine imino protons. This result reveals that C°G°dTdAC°G° indeed forms a self-complementary duplex, in which all the base residues are involved in hydrogen bonding. The signals abruptly broaden on increasing the temperature above 15 °C and finally disappear above 20 °C because of proton exchange with H_2O. This result suggests that the duplex is relatively unstable.

These imino proton resonances were assigned by nuclear Overhauser effect (NOE) experiments as shown in Figure 3. NOE arises from cross-relaxation between protons close in space and can be observed as a change in intensity of one signal upon irradiating another. It is known that an NOE between

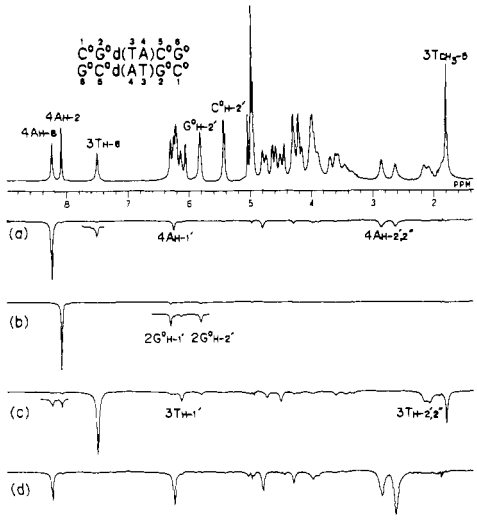

FIGURE 4: Nonexchangeable proton NMR spectra of C°G°dTdAC³G° (strand concentration, 5.7 mM) in D_2O containing 0.1 M NaCl, 0.01 M sodium phosphate buffer (pH 7.2), and 0.1 mM EDTA at 5 °C. A normal spectrum is shown at the top. (a–d) NOE difference spectra obtained by irradiating the 4dAH8 (a), 4dAH2 (b), 3dTH6 (c), and 4dAH2′ (d) proton signals.

the thymine imino proton and the adenine C2H proton of an A·T base pair and an NOE between the guanine imino proton and the cytosine amino protons of a G·C base pair can be easily observed (Gronenborn et al., 1984). Irradiation of the signal at 13.58 ppm produces NOE's to the signals at 8.41 and 7.08 ppm, which can be assumed to be cytosine amino proton signals (probably for the hydrogen-bonded proton and the free proton, respectively), but not to the adenine C2H signal (Figure 3a). Irradiation of the signal at 13.11 ppm gives a similar result (Figure 3b). These results suggest that these two imino proton signals are from the two guanine residues. The signal in the lowest field should be that of the terminal G° residue (6G°) since it is broader than the other and disappears earlier upon raising the temperature. It is found that the two signals at 8.41 and 7.08 ppm correspond to two sets of cytosine amino protons of 1C° and 5C°. Irradiation of one of these signals shows a large NOE on the other, suggesting that the corresponding protons belong to the same cytosine amino group (Figure 3d). Irradiation of the remaining imino proton signal at 12.13 ppm reveals an NOE on the adenine C2H, suggesting that the former is the thymine imino proton signal (Figure 3c). The minor NOE peak around 8.3 ppm may be that of the adenine amino proton(s). A minor NOE peak at the same position is also observed in the case of 2G° imino proton irradiation (Figure 3b). A molecular model building study shows that the 2G° imino proton can be close to the 4dA amino proton, which is hydrogen-bonded to the 3dT residue, of the opposite strand. An inter-base-pair NOE is observed between the imino protons of 6G° and 2G° (Figure 3a). These imino protons are especially close to each other in the molecular model.

Nonexchangeable Proton NMR Spectra. In order to obtain information concerning the glycosidic conformation and handedness of the d(TA) double-helical segment, NOE experiments for the nonexchangeable protons of C°G°dTdAC°G° were carried out in D_2O (Figure 4). The signals were assigned by decoupling and NOE experiments. When 4dAH8 is irradiated, NOE's, which are somewhat larger than that of 4dAH1′, are observed on the resonances of both C2′ protons of 4dA (Figure 4a). This result is consistent with a high anti glycosidic conformation of the 4dA residue. It should be noted that no NOE is observed on the 3dTH2′ and 3dTH2″ resonances. This result is not consistent with either the right-handed A-form or B-form structures (Gronenborn et al., 1984; Haasnoot et al., 1984). A very small NOE is observed on 3dTH6. This phenomenon is consistent with the left-handed stacking conformation. It is not observed for a pyrimidine–purine sequence in DNA (Hare et al., 1983) although an NOE between *n* purine H8 and *n* + 1 pyrimidine H5 is observed. Irradiation of the C2′ proton resonance inversely gives nearly equal NOE's to the 4dAH8 and 4dAH1′ resonances (Figure 4d). Irradiation of the 3dTH6 resonance gives nearly equal NOE's to both 3dTH2′ and 3dTH2″ resonances, which are larger than that of the 3dTH1′ signal (Figure 4c). This result suggests that the 3dT residue also takes a high anti glycosidic conformation. Small NOE's are also observed on both 4dAH8 and 4dAH2 resonances upon irradiation of 3dTH6. This result is consistent with a high anti left-handed structure but not with right-handed A- and B-form structures. Irradiation of the 4dAH2 resonance gives small NOE's to the 2G°H1′ and 2G°H2′ resonances (Figure 4b). These 2G° protons should be those of the 2G° residue in the opposite strand. These interstrand NOE's are again consistent with a high anti left-handed structure but not with right-handed A- and B-form structures (Patel et al., 1986). Moreover, a very small NOE is observed between the 4dAH2 and H1′ of the 5′ adjacent nucleoside residue (3dT) (Figure 4b). This phenomenon can be explained by the left-handed structure but not in terms of the right-handed structures (Patel et al., 1986).

Model of the High Anti Left-Handed Double Helix. A molecular model building study suggests that a combination of –guache, trans conformations about the O3′–P and P–O5′ bonds, trans conformation about the C5′–C4′ bond (IUPAC–IUB Joint Commission on Biochemical Nomenclature, 1983), and C3′-endo sugar puckering conformation gives a regular left-handed double helix with about 12 base pairs per turn for C°G°C°G°C°G° (Uesugi et al., 1985). This combination is in accordance with the results of an energy calculation study on a cyclonucleotide polymer by Fujii et al. (1976) and of a helical parameter analysis for polynucleotides by Yathindra and Sundaralingam (1976).

In order to visualize the left-handed structure of C°G°dTdAC°G°, the molecular mechanics calculations were carried out. The most preferable structure is shown in Figure 5. The conformation of the d(TA) segment of this model also conserves the left-handed helicity, and the whole structure of the hybrid duplex is similar to that of the C°G°C°G°C°G° duplex. The stacking pattern of the d(TA) step is shown in Figure 6. The helical twist of this pyrimidine–purine step is −42°, and this value is similar to those of other pyrimidine–purine steps in this structure. On the other hand, the average twist angle of the purine–pyrimidine step is −31°, resulting in a helix with about 10 base pairs per turn. In this structure, it should be noted that torsion angles about the O3′-P bond are mostly in the low –gauche region and furanose puckering forms are mostly C4′-exo. Although further adjustment in detail is needed to satisfy all the NOE data, this structure gives us an idea of what the left-handed duplex looks like. It should be noted that a high anti left-handed double helix has a shallow minor groove and a deep major groove similar to the right-handed A-form structure (Uesugi et al., 1985).

Discussion

NMR studies on C°G°dTdAC°G° clearly demonstrate that the hexamer forms a double helix with complete base pairing of the Watson–Crick type. The chemical shift (12.13 ppm at 1 °C) of the hydrogen-bonded dT imino proton is quite small for an A·T base pair (Gronenborn et al., 1984; Mirau

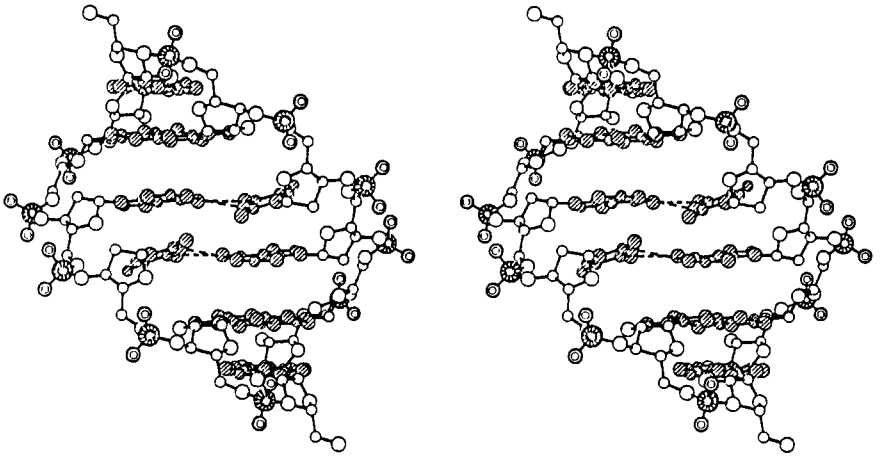

FIGURE 5: Stereoviews of an energy-minimized conformational model for C°G°dTdAC°G° from the direction of the minor groove.

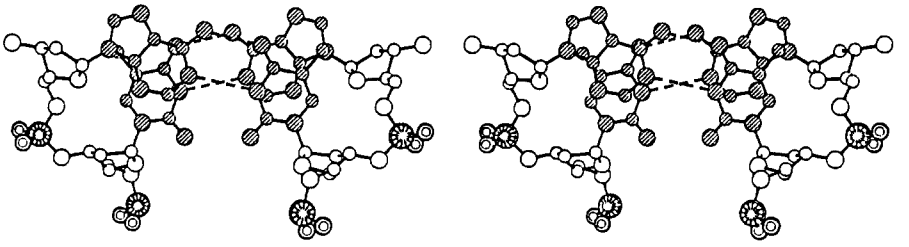

FIGURE 6: Stereoviews of a stacking pattern of the d(TA) segment in the conformational model of C°G°dTdAC°G°.

& Kearns, 1985). The reason for this unusual upfield shift is not known at present. Some strain in the base pairing, such as that caused by a propeller twist, might weaken the hydrogen bonds, increase the imino proton exchange rate with solvent protons, and cause an upfield shift of the resonance. Chemical shifts as small as 12.1 ppm are also observed for hydrogen-bonded dT imino protons at 4 °C in a deoxyribo-heptadecanucleotide duplex (Lee et al., 1985).

The results of NOE experiments between nonexchangeable protons strongly suggest that the deoxynucleoside residues are indeed incorporated into a part of a high anti left-handed double helix. Most of the NOE data can be explained, at least qualitatively, by the model obtained by energy calculation (Figure 5). The only difficulties remaining are for the glycosidic (χ = 88° for dT, χ = 102° for dA; Sundaralingam's definition) and sugar puckering (C3′-endo for dT, C4′-exo for dA) conformations. At present our assumption is as follows: To accommodate the somewhat unfavorable high anti glycosidic conformation, the deoxynucleoside residue may prefer an O1′-endo or C2′-endo furanose ring conformation, where the base residue is in a quasi-equatorial position to avoid steric repulsion between H8 or H6 and H2′. The former conformation seems to be favorable for stacking. This sugar conformation enables the base proton to come to a position (χ > 120°) nearly equidistant from both H2′ and H2″, which is in accordance with the NOE results.

We have also synthesized C°G°dCdGC°G° and examined its conformation by NMR spectroscopy (Uesugi et al., 1985). It appears that this hybrid hexamer also forms a high anti left-handed double helix. However, the evidence was not sufficiently conclusive since it contains three imino protons for guanine residues that have no H2 proton and the NMR spectra include signals of a minor conformational isomer. C°G°dCdGC°G° gives NOE results similar to those observed for C°G°dTdAC°G° upon irradiation of 4dGH8 or 3dCH6, including a small NOE between 4dGH8 and 3dCH6. In addition, a small NOE is also observed between 2G°H2′ and 3dCH1′.

The characteristic NOE's for the high anti left-handed double-helical structure described above are attributed to the unique mode of stacking (Uesugi et al., 1977; Fujii & Tomita, 1976), which is different from not only those of ordinary right-handed structures but also from that of the left-handed Z-form structure. We consider the d(TA) segment, which is a part of C°G°dTdAC°G°, and take the dT residue (5′-terminal residue) as a reference, assuming that it is fixed in the plane of the paper as seen in Figure 1 (see also Figure 6). In the ordinary right-handed helix, the adenine of the dA residue (3′-terminal residue) stacks above and to the right-hand side of the dT residue (Dickerson & Drew, 1981). In the high anti left-handed helix, the adenine of the dA residue stacks above and to the left-hand side of the dT residue (see Figure 6). Therefore, when a polynucleotide chain is extended in a 5′ → 3′ direction, a helix is formed above the plane of the paper in both cases but with a different handedness. In the case of the Z-form helix, the adenine of the dA residue stacks below and to the left-hand side of the dT residue and the next dT residue stacks below but to the right-hand side of the dA residue (Wang et al., 1979, 1984). When a polynucleotide chain is extended in the 5′ → 3′ direction, a left-handed helix is formed below the plane of the paper. This is the reason why the base-pair plane must be flipped over in a B → Z transition (Wang et al., 1979; Harvey, 1983).

The present study on C°G°dTdAC°G° and a similar study on C°G°dCdGC°G° (Uesugi et al., 1985) strongly suggest that DNA can form a high anti left-handed double helix under some constrained conditions as observed in the case of Z-DNA (Rich et al., 1984). We may call such a structure the H-form left-handed double helix to distinguish it from the Z-form helix and the left-handed helices with low anti glycosidic conformation as postulated by Sasisekharan et al. (Gupta et al., 1980). To accomplish a B → H transition, the right-handed helix is only required to unwind to a neutral position and then to rewind in the opposite direction without inversion of the base-pair plane and flipping over of the sugar residue (Harvey, 1983).

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