exceeds the amount of catalytic material (Pd or polymer) by a significant factor (over 1000 molecules of HCO_2^- per Pd atom added have been obtained). Also, the rate of reduction of CO_3H^- is slow, but the highest concentration of HCO_2^- produced is significant, up to 0.25 M. Producing higher concentrations of HCO_2^- should be possible, particularly if a better charge carrier than the $[(PQ^{2+/+})_n]$ can be found. The most negative potentials used allow reduction of the polymer partially to the $[(PQ^0)_n]$ state that is much less durable in aqueous electrolyte than is the $[(PQ^+)_n]$ state.[8,9] Additionally, the rate could be improved, since Pd on C has been shown[6] to be more active than the Pd-impregnated $[(PQ^{2+})_n]$ when using H_2 as the reductant. Even as the results stand, the current density of $\sim 100\ \mu A/cm^2$ for reduction of CO_3H^- to HCO_2^- near the thermodynamic potential shows that C–H bond formation from a CO_2 equivalent can occur electrochemically under mild conditions. As in the catalyzed reaction of H_2 with CO_3H^-, we find that under the same conditions where Pd-impregnated $[(PQ^{2+})_n]$ is an effective catalyst, the analogous Pt system has negligible activity. Spectroscopic studies of catalytic electrodes before and after use are under way to establish possible reasons for the differences between the Pt and Pd.

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Registry No. I, 74173-49-2; $PdCl_4^{2-}$, 14349-67-8; W, 7440-33-7; Pt, 7440-06-4; Pd, 7440-05-3; CO_3H^-, 71-52-3; HCO_2^-, 71-47-6.

Ribooligonucleotides, r(C-G-C-G) Analogues Containing 8-Substituted Guanosine Residues, Form Left-Handed Duplexes with Z-Form-like Structure

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The discovery of a left-handed DNA duplex structure (Z DNA) in d(C-G-C-G-C-G) crystals[1] has generated a great deal of interest in oligo- and polydeoxyribonucleotides containing alternating pyrimidine–purine sequences. In Z DNA, the dG residues adopt an unusual syn conformation about the glycosidic bond with C3′-endo furanose ring puckering while the dC residues take an anti and C2′-endo forms similar to those in B DNA. Oligo[d(C-G)]'s adopt the Z-form structure in solution at high salt concentrations.[2,3] However, corresponding ribooligonucleotides, oligo[r(C-G)]'s, do not take the Z-form structure under similar conditions.[4] Recently, we have suggested the possible existence of Z-form structure in RNA duplexes by conformational study of a r(C-G) analogue[5] containing 8-bromoguanosine (br^8G), which tends to adopt the syn conformation.[6,7] For characterization of Z RNA duplexes, however, the chain length ought to be at least four because the repeating unit in the Z-form structure is a dinucleotide.

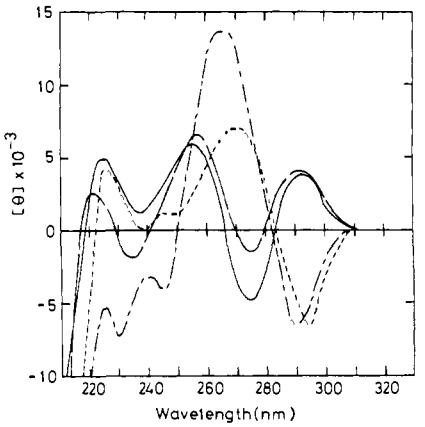

Figure 1. CD spectra of r(C-br^{8}G-C-br^{8}G)(—), r(C-m^{8}G-C-m^{8}G)(– –), r(C-G-C-G)(– · –), and d(C-G-C-G-C-G)(– – –) at 0 °C. The ribotetramers (1 A_{260} unit/mL) were measured in 0.1 M NaCl, 0.01 M phosphate buffer (pH 7.5). The deoxyhexamer (1 A_{260} unit/mL) was measured in 4 M NaCl, 0.01 M phosphate buffer (pH 7.5).

In this communication, we report the synthesis and characterization of r(C-br^{8}G-C-br^{8}G) and r(C-m^{8}G-C-m^{8}G). 8-Methylguanosine (m^8G) also has a tendency to adopt the syn conformation.[8] Studies on the tetramers by UV, CD, and ^1H NMR spectroscopy reveal that they form duplexes similar to that of Z DNA as observed for oligo[d(C-G)]'s in high salt solutions.[3]

r(C-br^{8}G-C-br^{8}G) and r(C-m^{8}G-C-m^{8}G) were synthesized by a phosphotriester method.[9,10] r(C-br^{8}G-C-br^{8}G) was synthesized by a dimer block condensation, and r(C-m^{8}G-C-m^{8}G) was synthesized by a stepwise condensation method. The tetramers obtained could be completely hydrolyzed by nuclease P1 to give C, pbr^{8}G (or pm^{8}G), and pC in 1:2:1 ratio.

The CD spectra of the modified tetramers at 1 °C are shown in Figure 1. The spectra of d(C-G-C-G-C-G) in 4 M NaCl and of r(C-G-C-G) in 0.1 M NaCl are also included. All the oligomers here were shown to form duplexes under the conditions in Figure 1 by UV and CD spectroscopy.[11] The r(C-G-C-G) analogues give spectra very similar in pattern and magnitude (λ_{max}'s at around 225, 255, and 290 nm and λ_{min}'s at around 235 and 275 nm).[12] Their CD patterns in the 260–310-nm region are quite different from that of d(C-G-C-G-C-G) in 4 M NaCl where the Z DNA structure is adopted. However, the CD spectrum of r(C-G-C-G), which has been proved to take an A-form structure,[4] is similar to that of d(C-G-C-G-C-G) in 4 M NaCl in this region. The clear inversion of signs of the 290-nm bands observed between r(C-G-C-G) and its 8-substituted derivatives strongly suggests that the modified tetramers may form left-handed duplexes.

In order to elucidate the conformations of the modified tetramer duplexes, ^1H NMR spectra were measured at various temperatures. Resonances of the base protons and some of the sugar protons were assigned by extensive ^1H–^1H decoupling and nuclear Overhauser effect (NOE) experiments. r(C-br^{8}G-C-br^{8}G) and r(C-m^{8}G-C-m^{8}G) showed similar chemical shift–temperature profiles for each proton suggesting that both tetramers have similar duplex structures.[13] NOE experiments on the m^{8}G-containing

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(12) CD spectral patterns of the modified tetramers in 4 M NaCl were similar to those in 0.1 M NaCl. T_m's of the modified tetramer duplexes increased with increasing salt concentration (at least up to 4 M NaCl).

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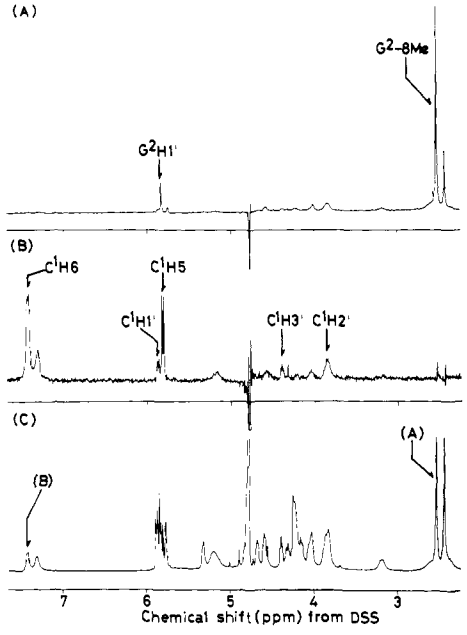

Figure 2. ^{1}H NMR spectrum (360 MHz) and NOE difference spectra of r(C-m^{8}G-C-m^{8}G) (14 mM) in 0.1 M NaCl, 0.01 M phosphate buffer (pD 7.5) at 20 °C. The chemical shifts were measured downfield from DSS. A single-frequency preirradiation pulse was applied for 0.3 s prior to data acquisition with 0.8-ms delay. (A) Irradiation at the methyl proton signal of the m^{8}G^2 residue, (B) irradiation at the H6 signal of the C^1 residue, (C) without irradiation. A negative NOE is observed as a positive peak.

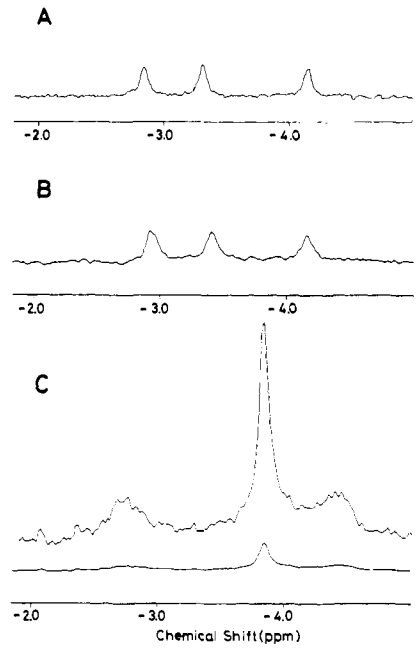

Figure 3. Totally proton-decoupled ^{31}P NMR spectra (145.8 MHz) of (A) r(C-m^{8}G-C-m^{8}G) (14 mM), (B) r(C-br^{8}G-C-br^{8}G) (9.4 mM) in 0.1 M NaCl, 0.01 M phosphate buffer (pD 7.5) at 5 °C, and (C) d(C-G-C-G) (16 mM) in 4 M NaCl, 0.01 M phosphate buffer (pD 7.5) at 20 °C. The chemical shifts were measured relative to external trimethyl phosphate.

tetramer can provide information about the glycosidic conformations of both m^{8}G and C residues. Weak irradiation of the 8-methyl protons of the first m^{8}G residue (m^{8}G^{2}CH$_3$) gives a specific NOE at a H1′ signal of the same residue (m^{8}G^{2}H1′) (Figure 2A) suggesting that this residue indeed takes a syn conformation. A similar result was also obtained between m^{8}G^{4}CH$_3$ and m^{8}G^{4}H1′. Irradiation of H6 of the first C residue (C^{1}H6) gives the largest NOE at C^{1}H5 and smaller NOE's at C^{1}H2′ and C^{1}H3′ as well as at C^{1}H1′ (Figure 2B). Irradiation of C^{1}H3′ inversely gave a small NOE at C^{1}H6. Similar results were also obtained between C^{3}H6 and the corresponding sugar protons. These results are consitent with an anti conformation of the C residues but not with a syn conformation. A similar weak NOE between CH6 and CH1′ has been observed for C residues in A RNA[4] and B DNA[14,15] duplexes. Moreover, coupling constants between H1′ and H2′ ($J_{1'2'}$) for both tetramers (6.8, ≤1, 6.8, and 2.9 Hz for C^1, br^{8}G^2, C^3, and br^{8}G^4, respectively, at 9.4 mM and 27 °C; 7.8, ≤1, 8.0, and 4.7 Hz for C^1, m^{8}G^2, C^3, and m^{8}G^4, respectively, at 14 mM and 25 °C) suggest that the C residues prefer C2′-endo sugar puckering while the br^{8}G or m^{8}G residues prefer C3′-endo sugar puckering.[16] These results clearly demonstrate that the cytidine residues and guanosine residues take alternating conformations about the glycosidic bonds and furanose rings as observed in Z DNA.

Another property characteristic of Z DNA is that it shows widely separated ^{31}P NMR signals (by about 1.5 ppm).[17,18] This is assumed to be due to the different conformations about the P–O bonds of d(G-C) (gauche$^-$–trans) and d(C-G) (gauche$^+$–gauche$^+$) fragments.[19] ^{31}P NMR spectrum of the 8-substituted r(C-G-C-G) derivatives (9.4 and 14 mM) shows widely separated peaks (the greatest chemical shift difference between the signals is 1.23 and 1.31 ppm for br^{8}G- and m^{8}G-containing tetramers, respectively, at 5 °C) (Figure 3B and 3A) while d(C-G-C-G) in 4 M NaCl (16 mM) shows 1.66 ppm separation of signals appearing at the highest and lowest fields at 20 °C (Figure 3C).[20]

In conclusion, the present results strongly suggest that r(C-br^{8}G-C-br^{8}G) and r(C-m^{8}G-C-m^{8}G) form Z-form-like RNA duplexes.[21,22] These results also suggest that oligo(C-G) sequences in natural RNA could form left-handed duplexes with the aid of some factor such as a protein,[23] salt,[24] or supercoiling,[25] which either destabilized the A RNA or stabilized the Z RNA structure as observed in the case of DNA.[26]

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(13) It was noted that some of the H5′ resonances, probably of the C residues, for both tetramers show unusually large upfield shifts (≈1.5 ppm) upon duplex formation. This phenomenon is consistent with the Z-form structure as the CH5′ protons are located in the close proximity of the next (3′-side) guanine rings in Z DNA.

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(20) The three peaks observed for these duplexes may correspond to the three phosphate groups in the tetramer. The appearance of a middle peak may be due to the end effect of a short oligonucleotide.

(21) r(C-br^{8}G-C-br^{8}G) crystallized in hexagonal lattice with cell dimensions $a = b = 32.05$ Å, $c = 42.70$ Å and $\gamma = 120°$. These values are similar to those of d(C-G-C-G) crystals: $a = b = 31.25$ Å, $c = 44.06$ Å, $\gamma = 120°$ (ref 22). The intensity distribution of X-ray diffraction also showed that the molecules are packed in a similar form.

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