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2′,5′-DNA Containing Guanine and Cytosine Forms Stable Duplexes

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Abstract: 2′,5′-Linked DNA oligomers bearing the bases guanine and cytosine are found to associate. Three 2′,5′-DNA strands composed entirely of the bases guanine and cytosine, two mutually complementary dodecamers, and a self-complementary octamer were prepared. Melting temperatures and free energies of duplex formation were determined for these oligomers and their natural, 3′,5′-linked DNA counterparts. From these data it was seen that 2′,5′-DNA duplexes have free energies approximately half that of the corresponding natural DNA duplexes under high ionic strength conditions (1 M NaCl). 2′,5′-DNA strand association was also found to persist under conditions of low solution ionic strength. These observations are consistent with 2′,5′-linked DNA comprising an alternative genetic material. A model is proposed where present-day 3′,5′-linked nucleotides arose by "genetic takeover" of 2′,5′-linked nucleotides.

Introduction

3′,5′-Linked polynucleotides encode genetic information in all organisms. While such polymers are eminently suited for biotic information storage, their suitability for a similar prebiotic role is open to question, as constraints imposed on replicating systems by biotic and prebiotic environments are very different. A variety of nucleic acid-like structures possibly present on the primitive Earth have been characterized in terms of molecular recognition properties, and some have been identified as possible ancestors of modern polynucleotides.[1,2] Recently, we[3] and others[4] have reported on the ability of nucleic acids bearing 2′,5′-phosphodiester linkages to self-associate through complementary base-pairing, a property necessary for faithful replication. Previous work with 2′,5′-linked DNA has dealt only with the base pair formed between adenine and uracil/thymine, where association was noted only at high solution ionic strength.[3,4a] We now report that 2′,5′-DNA bearing guanine and cytosine also associates, and that association persists at low ionic strength. Our present findings taken with previous ones are consistent with 2′,5′-linked DNA comprising an alternative four-component genetic material.

Results

Three 2′,5′-DNA strands, two mutually complementary dodecamers **3** and **4**, and a self-complementary octamer **7** (Table 1), composed entirely of the bases guanine and cytosine, were prepared using phosphoramidites **1** and **2** (Figure 1). After deprotection and purification, oligomers were characterized by digestion to constituent nucleosides, PAGE, and laser desorption time-of-flight mass spectrometry.

The ability of 2′,5′-DNA strands to associate was determined from profiles of UV absorbance versus temperature. Thus, a mixture of mutually complementary 2′,5′-DNA dodecamers **3** and **4** exhibits a sigmoidal absorbance versus temperature profile similar in character to the corresponding natural 3′,5′-linked DNA dodecamers **5** and **6** in 1 M aqueous NaCl (Figure 2A). Under

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(1) See, for example: (a) Eschenmoser, A. *Pure Appl. Chem.* **1993**, *65*, 1179. (b) Schneider, K. C.; Benner, S. A. *J. Am. Chem. Soc.* **1990**, *112*, 453. (c) Visscher, J.; Bakker, C. G.; van der Woerd, R.; Schwartz, A. W. *Science* **1989**, *244*, 329. (d) Schwartz, A. W.; Orgel, L. E. *Science* **1985**, *228*, 585.

(2) Joyce, G. F.; Schwartz, A. W.; Miller, S. L.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4398.

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Table 1. Thermodynamic Properties of Oligodeoxynucleotide Duplexes

	1 M NaCl[a]		0.1 M NaCl[b]	
oligodeoxynucleotide	T_m (°C)	$-\Delta G°_{37}$ (kcal/mol)	T_m (°C)	$-\Delta G°_{37}$ (kcal/mol)
5′-CCGGCCGCGCGC-2′ (3) 2′-GGCCGGCGCGCG-5′ (4)	42.4[c]	9.44	36.3[c]	8.25
5′-CCGGCCGCGCGC-3′ (5) 3′-GGCCGGCGCGCG-5′ (6)	76.8[c]	19.52	72.3[c]	18.47
5′-CGGCGCCG-2′ (7)	42.3[d]	6.90		
5′-CGGCGCCG-3′ (8)	66.9[d]	11.64		

[a] Sample contained 1 M NaCl, 10 mM sodium phosphate, 0.1 mM EDTA at pH 7. [b] Same conditions as in footnote a except 0.1 M NaCl was used in place of 1 M NaCl. [c] 5 μM total concentration of oligonucleotides. [d] 46 μM total concentration of oligonucleotides.

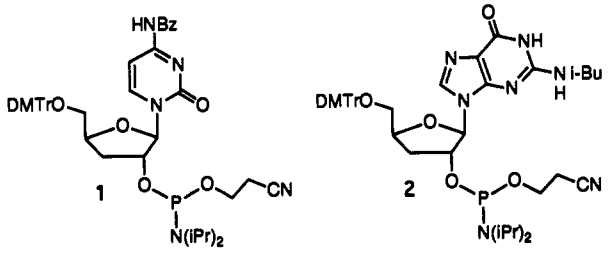

Figure 1.

the same salt conditions, self-complementary 2′,5′-DNA octamer 7 likewise gives a sigmoidal absorbance profile that is comparable to the corresponding natural oligomer (Figure 2B). Hyperchromicity observed in the absorbance of 2′,5′-DNA strands 3/4 and 7 with increasing temperature is consistent with double-strand complex formation.

Thermodynamic properties of 2′,5′-DNA and natural DNA complexes are summarized in Table 1. Melting temperatures and free energies of duplex formation were determined by nonlinear regression fitting of melting curves with a bimolecular, two-state model.[5] From these data it is seen that 2′,5′-DNA duplexes derived from 3/4 and 7 have free energies approximately half that of the corresponding natural DNA duplexes under high ionic strength conditions (1 M NaCl).

Bimolecular association of 2′,5′-DNA oligomers 3/4 and 7 is supported by concentration dependencies of melting temperatures. Hence, a 28-fold increase in the concentration of 3/4 (5 to 140 μM) under 1 M salt conditions gave an 11.0 °C increase in melting temperature. This corresponds to a $-\Delta G°_{37}$ for duplex formation of 9.44 kcal/mol,[5,6] in agreement with the value given in Table 1 derived from nonlinear regression curve fitting. In similar fashion, a 3-fold enhancement in concentration of 7 (46 to 140 μM) led to a melting temperature increase of 5.6 °C, corresponding to a $-\Delta G°_{37}$ for duplex formation of 6.82 kcal/mol, in accord with Table 1 once again.

Finally, association of 2′,5′-DNA dodecamers 3 and 4 was found to persist under low ionic strength conditions (Table 1). Sensitivity of the 2′,5′-DNA duplex to a shift in [NaCl] from 1 to 0.1 M is observed to be similar to natural DNA duplex 5/6, as reflected in the free energy values.

Discussion

The thermodynamic stability of 2′,5′-DNA duplexes bearing guanine and cytosine is found to be approximately one-half that of 3′,5′-DNA. This stability difference is similar to that observed for 2′,5′- versus 3′,5′-RNA by Turner[4b] and is qualitatively consistent with our previous observations on 2′,5′-DNA bearing adenine and uracil.[3] These studies also give an indication of the specificity with which 2′,5′-linked nucleic acids base-pair. Evidence consistent with formation of two types of Watson–Crick base-pairs by 2′,5′-linked nucleotides has been observed by ourselves in this and previous work[3] and also by others.[4] Collectively, these findings permit 2′,5′-linked nucleotides to be considered an alternative genetic material.

Efficient abiotic replication of an informational macromolecule requires balancing of many, potentially contradictory, factors. For example, the high affinity that complementary 3′,5′-linked nucleotides have for one another, while beneficial for daughter strand assembly, also impedes replication by inhibiting the turnover of strands once such an assembly is complete.[7] The 2′,5′-linkage could conceivably have a selective advantage in abiotic replication as a result of the weaker self-affinity it imparts. If 2′,5′-linked nucleic acids (or some other informational polymer) were the most fit in a primitive environment, it is conceivable that the 3′,5′-linkage arose by "genetic takeover". That is, given a self-replicating polymer, a metabolism based on that polymer could have arisen that included a capability to synthesize 3′,5′-linked nucleic acids, leading ultimately to the taking over of all metabolic roles and also the part of genetic material by the latter.[2,8] Interestingly, evidence for mutual recognition between 2′,5′- and 3′,5′-linked nucleic acids has been reported.[9] Experiments are underway to explore further the possibility of an ancestral 2′,5′-linkage.

Experimental Section

General Methods. Controlled pore glass support was purchased from CPG, Inc. (Fairfield, NJ). Chemicals were purchased from either Sigma or Aldrich Chemical Co. Solvents were purchased from Fisher Scientific. T4 polynucleotide kinase was purchased from Boehringer Mannheim. 5′-(γ-^{32}P)ATP (~3000 Ci/mmol) was purchased from Amersham. CH_2Cl_2 was distilled from CaH_2 and stored over molecular sieves. ^{1}H NMR spectra were recorded on a GE QE-300 (300 MHz) spectrometer. ^{31}P NMR spectra were recorded on a GE GN-500 (202 MHz, H_3PO_4 external standard) spectrometer. Ultraviolet spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer. Mass spectra and high-resolution mass spectra were recorded on a VG-ZAB-2FHF mass spectrometer using FAB ionization at the Southern California Regional Mass Spectrometry Facility (University of California, Riverside). Laser desorption mass spectra were recorded on a Finnegan Lasermat mass spectrometer at the Biotechnology Instrumentation Facility (University of California, Riverside).

Synthesis, 4-*N*-Benzoyl-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (1). To a solution of 4-*N*-benzoyl-3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)cytidine[10] (430 mg, 0.679 mmol) in CH_2Cl_2 (5 mL) were added diisopropylethylamine (263 mg, 2.04 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphine (321.4 mg, 1.36 mmol) successively at room temperature. The reaction mixture was stirred for 30 min. Another 263 mg of diisopropylethylamine and 321 mg of 2-cyanoethyl *N*,*N*-diisopropylchlorophosphine were added in turn, and the mixture was stirred for an additional 30 min. The reaction was diluted with CH_2Cl_2 (50 mL) and washed with 5% aqueous $NaHCO_3$ (2 × 30 mL), and the organic layer was dried ($MgSO_4$). Flash column chromatography (SiO_2, EtOAc (33%)/triethylamine (1%)/CH_2Cl_2) gave 360 mg (64%) of the desired product as a mixture of two diastereomers. ^{1}H NMR ($CDCl_3$) δ: 1.15–1.30 (m, 24 H), 1.90–2.22 (m, 4 H), 2.60–2.80 (m, 4 H), 3.37–4.06 (m, 12 H), 3.83 (s, 12 H), 4.59–4.66 (m, 4 H), 6.02–6.05 (m, 2 H), 6.87–6.90 (m, 8 H), 7.16–7.70 (m, 28 H), 7.87 (m, 4 H), 8.56–8.61 (m, 2 H). ^{31}P NMR ($CDCl_3$) δ: 149.13, 151.33. MS (FAB$^+$) *m*/*z*: 834 (MH^+). HRMS (FAB$^+$): Calcd for $C_{46}H_{53}N_5O_8P_1$ 834.3632 (MH^+), found 834.3626.

3′-Deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2-*N*-isobutyrylguanosine 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (2). To a solution of 3′-deoxy-5′-*O*-(4,4′-dimethoxytrityl)-2-*N*-isobutyrylguanosine[10] (200 mg, 0.31 mmol) in CH_2Cl_2 (3 mL) were added diisopropylethylamine (120.2 mg,

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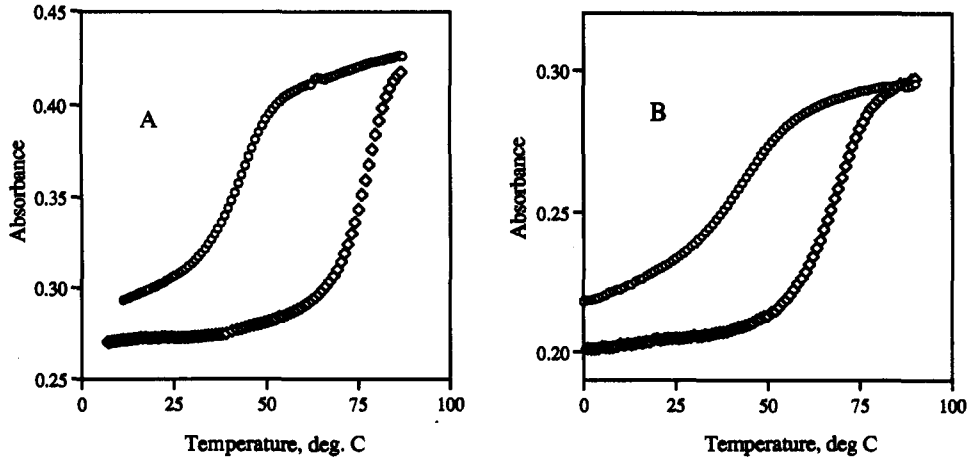

Figure 2. (A) UV absorbance profiles for 5'-CCGGCCGCGCGC-2' (**3**)/2'-GGCCGGCGCGCG-5' (**4**) (○) and 5'-CCGGCCGCGCGC-3' (**5**)/3'-GGCCGGCGCGCG-5' (**6**) (◇). Experimental conditions were as noted in footnotes *a* and *c* of Table 1; measurements were performed using a 1 cm path length quartz cell. (B) 5'-CGGCGCCG-2' (**7**) (○) and 5'-CGGCGCCG-3' (**8**) (◇). Experimental conditions were as noted in footnotes *a* and *d* of Table 1; measurements were performed using a 0.1 cm path length quartz cell.

0.93 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphine (146.7 mg, 0.62 mmol) successively at room temperature. The reaction mixture was stirred for 30 min. The mixture was then diluted with CH_2Cl_2 (50 mL) and washed with 5% aqueous $NaHCO_3$ (2 × 20 mL), and the organic layer was dried ($MgSO_4$). Flash column chromatography (SiO_2, EtOAc (25%)/triethylamine (1%)/CH_2Cl_2, then MeOH (1%)/triethylamine (1%)/CH_2Cl_2) gave 110 mg (42%) of the desired product as a mixture of two diastereomers and 140 mg of a mixture of the desired product and $HPO(OCH_2CH_2CN)N(iPr)_2$ as an inseparable impurity, in a ratio of 1:2. ^1H NMR ($CDCl_3$) δ: 1.01–1.39 (m, 36 H), 2.01–2.76 (m, 10 H), 3.22–3.92 (m, 12 H), 3.76 (s, 12 H), 4.59 (m, 2 H), 4.95 (m, 1 H), 5.37 (m, 1 H), 5.89 (s, 1 H), 6.02 (s, 1 H), 6.77–6.81 (m, 8 H), 7.16–7.45 (m, 18 H), 7.85 (s, 2 H), 8.70 (s, 1 H), 8.85 (s, 1 H). ^{31}P NMR ($CDCl_3$) δ: 149.65, 149.36. MS (FAB$^+$) *m/z*: 840 (MH^+). HRMS (FAB$^+$): calcd for $C_{44}H_{55}N_7O_8P_1$ 840.3849 (MH^+), found 840.3839.

DNA Synthesis. All oligodeoxynucleotides were synthesized trityl-on using a controlled pore glass solid support via the phosphite-triester method with an Applied Biosystems 391EP DNA synthesizer (1-μmol scale), deprotected, and purified by previously described methods.[11] For the synthesis of oligomers **3**, **4**, and **7**, controlled pore glass support derivatized with *N*-benzoyl-5'-(dimethoxytrityl)-3'-deoxycytidine and *N*-isobutyryl-5'-(dimethoxytrityl)-3'-deoxyguanosine was prepared by standard methodology.[12] Oligonucleotide purity was verified by end labeling with polynucleotide kinase and ATP followed by PAGE.[11] The extinction coefficients (at 260 nm) of the natural nucleotides used for all concentration calculations were as follows: dCMP, 7700, and dGMP, 11 500, and all oligonucleotides were quantified on the basis of the absorbance observed when heated to 70 °C. Base composition of oligomers **3**, **4**, and **7** was confirmed by digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase followed by HPLC comparison with samples of 3'-dC and 3'-dG purchased from Sigma Chemical Co. (Alltech, HS, C-18; 20 mM K_2HPO_4, pH 5.5 (A), MeOH (B), 100% A to 40% B, 20 min).[13] Laser desorption mass spectral data for oligonucleotides **3**, **4**, and **7** are as follows. 5'-CCGGCCGCGCGC-2' (**3**): calculated mass ($(M - 1H)^-$) 3607; observed mass 3607. 5'-GCGCGCGGCCGG-2' (**4**): calculated mass ($(M - 1H)^-$) 3687; observed mass 3687. 5'-CGGCGCCG-2' (**7**): calculated mass ($(M - 1H)^-$) 2411; observed mass 2412.

Melting Experiments. These experiments were performed as previously described with the exception that UV absorbance recorded at 280 nm was used in the calculation of T_m's and free energy values.[11]

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