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An Unusual Mode of DNA Duplex Association: Watson-Crick Interaction of All-Purine Deoxyribonucleic Acids

Thomas R. Battersby,[1,*] Maria Albalos,[1] and Michel J. Friesenhahn[1]
[1] Bayer HealthCare LLC, Diagnostics Division, 725 Potter Street, Berkeley, CA 94710, USA
*Correspondence: thomas.battersby.b@bayer.com
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SUMMARY

Nucleic acid duplexes associating through purine-purine base pairing have been constructed and characterized in a remarkable demonstration of nucleic acids with mixed sequence and a natural backbone in an alternative duplex structure. The antiparallel deoxyribose all-purine duplexes associate specifically through Watson-Crick pairing, violating the nucleobase size-complementarity pairing convention found in Nature. Sequence-specific recognition displayed by these structures makes the duplexes suitable, in principle, for information storage and replication fundamental to molecular evolution in all living organisms. All-purine duplexes can be formed through association of purines found in natural ribonucleosides. Key to the formation of these duplexes is the N_3-H tautomer of isoguanine, preferred in the duplex, but not in aqueous solution. The duplexes have relevance to evolution of the modern genetic code and can be used for molecular recognition of natural nucleic acids.

INTRODUCTION

All-purine systems capable of specific recognition of non-repeating nucleic acid sequences through Watson-Crick pairing have been proposed as predecessors of the modern genetic system [1, 2]. However, established association of all-purine nucleic acids has been limited to homopolynucleotides in triplex [3], tetraplex [4, 5], and non-Watson-Crick duplex [6, 7] structures, as well as repeating sequences forming unusual duplexes without sequential base pairing [8]. An early report of a Watson-Crick duplex between homopolynucleotides polyadenosine and polyinosine appeared [9], but it was subsequently shown that only a 1:2 complex is formed [10]. Duplexes with non-repeating sequence and duplexes associating through Watson-Crick interaction remain unknown among all-purine nucleic acids.

Using the stability of purine-purine mismatches within a canonical duplex as a guide [11], we have constructed all-purine duplexes associating through Watson-Crick pairing. The antiparallel deoxyribose duplexes lack pyrimidines and violate the size-complementarity principle of natural nucleobase pairing. We demonstrate that all-purine duplexes can be formed through association of purines found in natural ribonucleosides with nucleic acids bearing only guanine, adenine, hypoxanthine (H), and isoguanine (J) [12, 13] nucleobases.

RESULTS AND DISCUSSION

Specific pairing of all-purine duplexes was demonstrated with a series of oligodeoxyribonucleotides (ODNs) (Table 1). Initially, association of these ODNs was followed by monitoring the emission of excited 6-fluorescein (FAM) labels with changing temperature. Transitions between single strands and the complex were observed because quenching [14] of excited 5′-FAM by an intramolecular 3′-Black Hole Quencher (BHQ) is greater when a dual-labeled ODN is single stranded. Fluorescence monitoring of association was chosen for initial experiments instead of conventional UV spectroscopy for two reasons. First, detection of hyperchromicity related to novel duplex dissociation may not be straightforward; hyperchromicity displayed by single-stranded, purine-rich ODNs with increasing temperature is significant and could obscure hyperchromicity resulting from transitions between duplexes and single strands. Second, UV monitoring of association may be confounded by differences in tautomeric representation of isoguanine when in single-stranded or complexed states. Unlike conventional oligonucleotide duplexes, a regular increase in absorbance across wavelengths may not occur because shifts in the tautomeric ratio of constituent nucleobases cause changes in ODN absorbance profiles.

ODNs **1** and **2** were designed to form antiparallel duplex **1**·**2**, in which A is opposite H and G is opposite J (Figure 1). An all-purine ODN (**3**) not expected to form a stable duplex with **1** was also prepared. ODNs **1** and **2** demonstrated a clear temperature-dependent cooperative association, while no interaction was observed between **1** and **3** (Figure 2A). The possibility of duplex formation of **1** with a parallel-oriented ODN was excluded in a similar

Table 1. ODNs Used

1	5′-(FAM)JGGAGAAAGHJGHG(BHQ)-3′
2	3′-GJJHJHHHJAGJAJ-5′
3	3′-GAAGAGGGAAGAAA-5′
4	5′-JGGAGAAAGHJGHG-3′
5	5′-(FAM)JGGZGZAZGHJGHG(BHQ)-3′
6	5′-(FAM)JGHAHAAAHHJGHG(BHQ)-3′
7[a]	3′-TFCCTCTTTCCFCCC-5′
8	5′-(FAM)CGGAGAAAGTCGTG(BHQ)-3′
9	3′-GCCTCTTTCAGCAC-5′
10[a]	3′-TFCCTCTTTJAGJAJ-5′
11	5′-CGGAGAAAGTCGTG-3′
12	3′-GJJXJXXXJAGJAJ-5′

[a] A 3′ dangling T was added to allow for the use of a standard solid-phase in synthesis.

experiment (Figure S1; see the Supplemental Data available with this article online).

ODNs **1** and **2** associated in a 1:1 complex. Nondenaturing gel electrophoresis of a mixture of **1** and **2** yielded a single band migrating as expected for duplex formation (Figure 2B). Mixing curves [15] of **4** (ODN **1** lacking fluorophore and quencher) and **2** indicated a 1:1 complex (Figure 2C), as did an experiment monitoring fluorescence in which 0–1.75 equivalents of **2** were mixed with **1** (Figure 2D). Replacing all Na^+ in solution with Li^+ had a negligible effect on the association of **1**·**2** (data not shown). CD spectroscopy [15] also indicated association of **4** and **2** (Figure S2).

Interaction of additional all-purine ODNs with fluorescent labels supported Watson-Crick interaction in purine-purine association. Although **1**·**2** appears likely to arise through *anti-anti* Watson-Crick interaction of the constituent purines, *syn-anti* Hoogsteen interaction is possible for A·H and G·J pairs. In ODN **5**, three adenines of **1** were changed to 7-deazaadenine (Z). In ODN **6**, three guanines of **1** were changed to H. Obtaining significantly lower melting temperatures (T_ms, temperatures at which 50% strand dissociation occurs) for **5**·**2** than for **1**·**2** under identical conditions would imply *anti-syn* H·A association in **1**·**2** (Figures S3A and S3B). Alternatively, obtaining significantly lower T_ms for **6**·**2** than for **1**·**2** would imply *anti-anti* G·J association in **1**·**2** (Figures S3C–S3E). T_ms observed for **5**·**2** were quite similar to those of **1**·**2**, while **6**·**2** was clearly destabilized relative to **1**·**2**, suggesting Watson-Crick interaction (Figure 3).

Changes in absorbance spectra of **4**·**2** with temperature reveal a shift in the tautomeric composition of constituent J nucleobases that further supports Watson-Crick association. The N_1-H tautomer of J has a characteristic UV maximum near 295 nm, while the N_3-H tautomer of J required for Watson-Crick association with G absorbs somewhat less in this region [16, 17]. Absorbance profiles of **4**·**2** at 280–310 nm indicate that J shifts substantially to the N_1-H tautomer with increasing temperature (Figure 4). Absorbance profiles of individual ODNs **4** or **2** display lesser tautomeric shifts under identical conditions (Figure S4). Conversely, absorbance profiles of duplexes associating through purine-pyrimidine interaction with J opposite 5-methylisocytosine (F) [18, 19], a pyrimidine Watson-Crick complement of the N_1-H tautomer of J, show a decrease in N_1-H tautomer as the duplexes dissociate with increasing temperature (Figure S5).

In contrast to the established N_1-H tautomer of J observed opposite F within conventional nucleic acid duplexes, formation of **4**·**2** and changes in the associated absorbance spectra are consistent with J opposite G in this duplex adopting the N_3-H tautomer. Although not described in studies of the tautomerism of isoguanine [16, 17], existence of the N_3-H tautomer is in accordance with the conclusions of both Eschenmoser and coworkers in extensive experiments changing backbone sugars in nucleic acids [20, 21] and a computational study by Switzer and coworkers [22]. While additional sequence contexts must be examined, our work suggests that a mixture of N_1-H and N_3-H tautomers of J exists in single-stranded ODNs in solution, and that tautomeric composition depends on sequence and temperature. The existence of some N_3-H tautomer of J in single-stranded ODNs explains recent work [23] in polymerase replication of ODNs containing F and J. During replication near room temperature, a fraction of template J positions did not direct incorporation of F or any natural nucleotides, consistent with template N_3-H tautomer.

The association of **1**·**2** was weaker than that of comparable duplexes formed through canonical purine-pyrimidine interaction. Additional ODNs **7**–**9** were used to create two control duplexes associating through Watson-Crick purine-pyrimidine interaction. All-pyrimidine ODNs **7** and **1** can form polypurine-polypyrimidine duplex **1**·**7**. In **8**·**9**, A·H and G·J pairs of **1**·**2** were changed to A·T and G·C pairs, respectively, giving a canonical duplex with identical interstrand hydrogen-bonding patterns. T_ms were substantially lower for **1**·**2** than for the two control duplexes (Figure 3). Chimeric duplex **1**·**10**, associating through eight consecutive purine-pyrimidine pairs and six

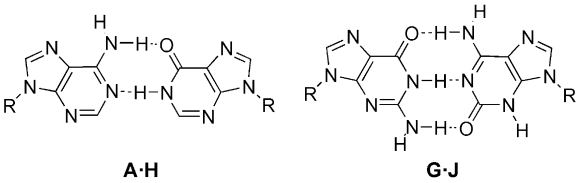

Figure 1. Watson-Crick Purine-Purine Pairs Used
Adenine and hypoxanthine, the nucleobase portion of inosine, can straightforwardly form a Watson-Crick pair, defined here as an *anti-anti* pair in which the hexagons of the six-membered purine rings align with N_1 directly opposite N_1', creating a pseudo two-fold axis in the plane of the ring systems. Guanine and isoguanine can form a Watson-Crick pair only with J as the N_3-H tautomer. The N_1-H tautomer of isoguanosine, however, predominates in aqueous solution.

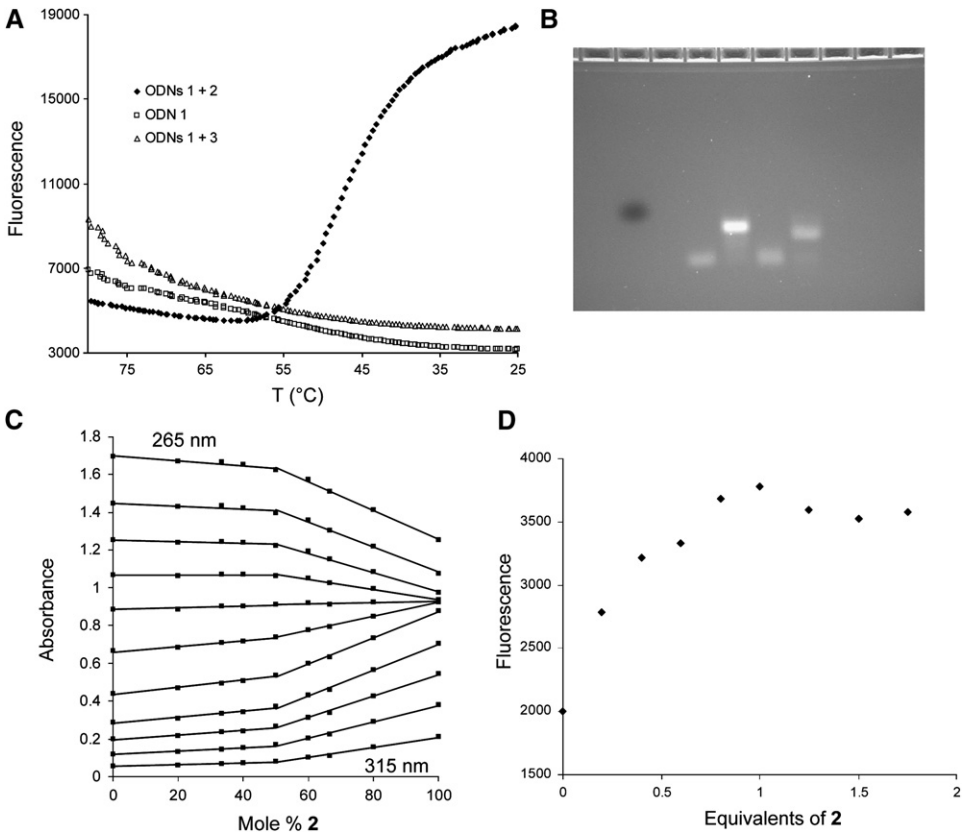

Figure 2. Association of All-Purine ODNs

(A) ODNs **1** and **2**, each at 1 μM, demonstrated a cooperative temperature-dependent association detected by monitoring the change in fluorescence of **1**. In contrast, **1** at 1 μM, or **1** and **3** each at 8 μM did not interact.

(B) The four lanes of the agarose gel with visible fluorescent bands contain, from left to right **8**, **8** and **9**, **1**, **1** and **2**. The shift in mobility of **1** when mixed with equimolar **2** resembles the shift in mobility of **8** when mixed with equimolar **9** to form canonical duplex **8**·**9**.

(C) In mixing curves of **4** and **2**, in which absorbance was monitored from 265 to 315 nm (5 nm intervals shown), a clear discontinuity at equimolar **4** and **2** is present, demonstrating a 1:1 complex.

(D) In a series of wells, 0–1.75 equivalents of **2** were mixed with **1**, and the resulting fluorescence of **1** was measured. Although **1** was at 125 nM in each well, fluorescence increased with increasing **2**, until 1 equivalent of **2** was added. Fluorescence then leveled off as the amount of **2** exceeded 1 equivalent, consistent with a 1:1 complex of **1** and **2**.

consecutive purine-purine pairs, had intermediate stability (Figure 3). Accommodation of the purine-purine structure in this chimeric duplex without destabilization suggests that the backbone of the duplex is not forced into an unfavorable conformation at the junction of the purine-pyrimidine and purine-purine regions.

Thermodynamic parameters were estimated with UV absorbance measurements for association of all-purine duplex **4**·**2** and for association of control canonical duplex **11**·**9**, an unlabeled analog of **8**·**9**. A ΔG°_{37} of −16.9 kcal/mol for association of **11**·**9** was determined from a van't Hoff plot of T_ms at 260 nm (Figure S6A). Although changes in absorbance of **4**·**2** upon dissociation arise at least partially from a shift in the tautomeric distribution of J, the changes are triggered by duplex dissociation, which mimics the hyperchromicity observed with canonical duplexes. Therefore, ΔG°_{37} of **4**·**2** association was roughly estimated as −10 kcal/mol by measuring T_ms at 248 and 303 nm (Figures S6B–S6D).

In contrast to all-purine duplexes, the association of "xDNA" duplexes of Kool and coworkers, with a C_1-C_1' distance of ~13 Å and a natural deoxyribose-phosphate backbone, is usually more favorable than association of corresponding DNA duplexes [24]. It is not immediately evident why xDNA duplexes and the all-purine duplexes, with presumed similar C_1-C_1' separation, have such a large difference in association. A possible explanation is that nucleobases opposite adenine in the all-purine duplexes do not position a carbonyl opposite C_2 of A. The absence of a carbonyl in this position could destabilize duplexes by disrupting hydration [25] or by removing a favorable electrostatic interaction [26] between an electronegative carbonyl oxygen and a partial positive charge at the edge of the opposing heterocycle. Some of the "missing" carbonyls opposite adenines in **1**·**12** were introduced by replacing four H nucleobases with 7-deazaxanthosine to give **1**·**12**. Duplex denaturation experiments with **1**·**12** demonstrated a stabilizing effect of these

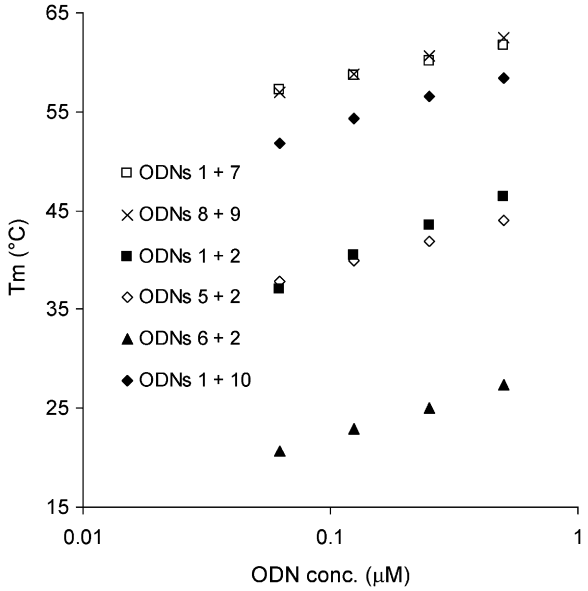

Figure 3. T_ms of Various Duplexes

T_ms determined by monitoring fluorescence of equimolar pairs of ODNs were plotted against log concentration of each strand. Replacing three H·A pairs in **1**·**2** with H·Z pairs (**5**·**2**) did not substantially change T_ms. Replacing three G·J pairs in **1**·**2** with H·J pairs (**6**·**2**) dramatically lowered T_ms. The relative T_ms of **5**·**2** and **6**·**2** are consistent with Watson-Crick interaction. Duplexes **1**·**7** and **8**·**9** with purine-pyrimidine interaction had higher T_ms than all-purine duplex **1**·**2**. Chimeric duplex **1**·**10**, with six consecutive purine-purine and eight consecutive purine-pyrimidine interactions, had T_ms between those of **1**·**2** and the duplexes associating through purine-pyrimidine interaction.

carbonyls (Figure S7), but the magnitude of the effect appears insufficient to account for the difference in association between **1**·**2** and **8**·**9**. The difference in the relative orientation of the bonds between nucleobase and C_1' may also destabilize all-purine duplexes relative to xDNA. In addition, individual electrostatic and hydrophobic interactions between adjacent paired nucleobases differ between xDNA and all-purine deoxyribose duplexes and are important in determining duplex stability.

It has been hypothesized that synthesis of complementary RNA from a template RNA molecule before the existence of modern biocatalysts was a stage in the origin of an ancient RNA world [27]. The early self-replicating system would have then evolved into the modern genetic system. All-purine ribonucleic acids have held some appeal as this self-replicating system for several reasons [1–3, 7, 8, 28, 29]. Ribose itself is a plausible product of prebiotic synthesis [30]. The origination of an all-purine system requires a more limited synthetic repertoire than a purine-pyrimidine system; the nucleobases in an all-purine system are plausible sibling products of analogous reaction pathways. Reactions to give pyrimidine ribonucleosides under putative prebiotic conditions have proved more elusive than the analogous reactions yielding purine ribonucleosides [27]. Purines have a higher propensity for self-organization than pyrimidines because of a larger surface area for stacking; in the absence of a catalyst, an all-purine system may, therefore, oligomerize more readily than a purine-pyrimidine system [2].

Despite much conjecture about the formation of sequence-specific all-purine duplexes, our experiments provide the first, to our knowledge, clear evidence of such duplexes with a natural backbone. Considering the relatively undistorted backbone of the Watson-Crick duplex contained within polyadenosine-polyinosine triplexes [3], it will not be surprising to find that ribonucleic acids also form analogous all-purine duplexes. Proposals of a precursor all-purine system can thus be reconsidered with the nucleobase components now demonstrated to form sequence-specific duplexes. A two-component system of H and A that originally inspired speculation [1] about all-purine precursors cannot form stable

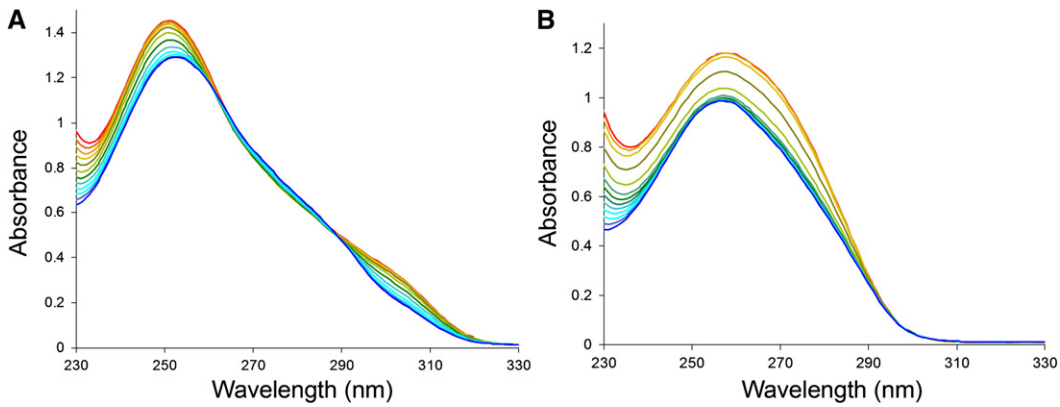

Figure 4. Overlaid Absorbance Spectra of All-Purine and Canonical Duplexes with Changing Temperature

(A) Absorbance spectra of **4**·**2** varied with temperature in a complex way (5°C increments shown). Spectra of **4**·**2** (4 μM of each ODN) displayed a greater than 50% hyperchromicity with increasing temperature near 300 nm, consistent with a shift in the tautomeric composition of J to N_1-H. Two regions displaying hyperchromicity, and potentially suitable for T_m determination, were observed near 250 and 300 nm.

(B) In contrast, spectra of **11**·**9** (4 μM of each ODN) displayed a sharper transition and a broad range of wavelengths suitable for T_m measurement.

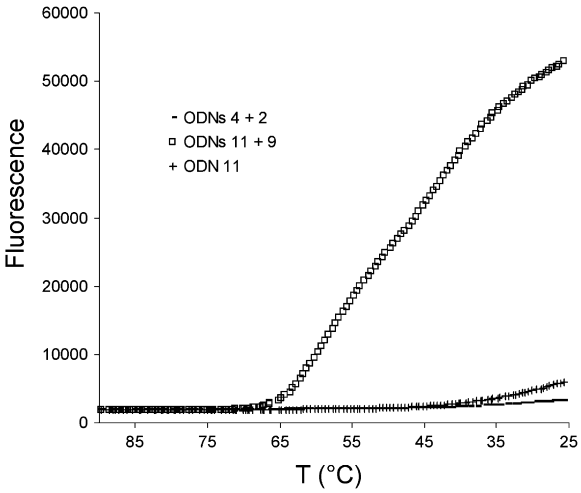

Figure 5. Lack of SYBR Green I Dye Fluorescence in the Presence of an All-Purine Duplex

Fluorescence increased as ODNs **11** and **9** annealed with decreasing temperature to form a canonical duplex in the presence of SYBR Green I. ODN **9** alone in the presence of SYBR Green I exhibited very little increase in fluorescence with decreasing temperature. As ODNs **4** and **2** formed an all-purine duplex with decreasing temperature, no increase in fluorescence was apparent in the presence of SYBR Green I.

Watson-Crick nucleic acid duplexes [7, 8, 10]. Nucleic acids composed of G and J with a natural backbone may form stable duplexes, although competing complexes in the presence of common cations [4, 5] may be a significant impediment. A four-purine system of A, H, G, and J, while not as discriminating as the modern recognition system, is still perhaps more accessible through prebiotic synthesis. However, it is unclear if the tautomeric ambiguity of J would allow the necessary template-directed polymerization. It is also not evident how a system employing J would transition to the present genetic code. Such a transition could possibly involve chimeric structures composed of purine-purine and purine-pyrimidine nucleobase pairs. Introduction of T to establish A·T pairs before the introduction of C into the structures could help avoid ambiguity, as C is a Watson-Crick complement to both G and H. Further work is required to ascertain if apposite transitional duplex structures are feasible.

Finally, all-purine duplexes will have novel properties and should have useful technological applications. Natural nucleic acid sequences can be targeted with probes forming all-purine or chimeric purine-purine/purine-pyrimidine interactions. All-purine nucleic acids can form duplex structures that will not act as substrates in many enzymatically mediated processes, such as polymerase replication, ligation, restriction, and mismatch repair. Small molecules used to target duplex nucleic acids will also exhibit changed interaction with all-purine duplexes. This allows for, for example, the formation of all-purine duplexes in the presence of SYBR Green I dye [31] without an increase in fluorescence above the level observed for single-stranded ODNs (Figure 5).

SIGNIFICANCE

Association of the all-purine duplexes is remarkable among complexes of deoxyribo- or ribonucleic acids. To our knowledge, this is the first demonstration of mixed-sequence nucleic acids with a natural backbone associating in a duplex structure, other than the canonical Watson-Crick structure. The all-purine duplexes demonstrate that pyrimidine nucleobases are not required for sequence-specific formation of a deoxyribose duplex, a structure vital for the replication of nucleic acids. The sequence-specific recognition demonstrated by these structures makes the duplexes suitable, in principle, for information storage and replication by a mechanism analogous to the genetic system. The duplexes have unique properties and interact with enzymes and small molecules quite differently than duplexes associating through purine-pyrimidine interaction. All-purine duplexes in which one strand is derived wholly from the modern genetic code are possible, as are chimeric duplexes containing regions of purine-pyrimidine and purine-purine interaction.

Molecular recognition by all-purine duplexes has relevance to the evolution of the genetic system. Even if all-purine duplexes played no direct role in the evolution of modern nucleic acids, consideration of their existence is important for understanding what constitutes biological fitness among nucleic acids. All-purine duplexes, which can be constructed entirely from purines found in natural ribonucleosides, may have been functionally selected against, and not merely absent as a result of synthetic contingency. Compared to other hypothesized competitors to the natural pairing system [32], there is less likelihood of a synthetic barrier to generating these all-purine duplexes.

EXPERIMENTAL PROCEDURES

All ODN association experiments were conducted in 0.045 M sodium citrate, 0.45 M sodium chloride (pH 7.9) solutions unless otherwise indicated. Data from denaturation experiments were fitted in S-Plus (Insightful) by using cubic smoothing splines [33]. T_ms were determined as the maximum of the first derivative of the fitted curve.

Oligodeoxyribonucleotides

ODNs containing inosine, 7-deazaadenosine, 7-deazaxanthosine, 5-methylisocytidine, or isoguanosine were prepared from phosphoramidites (Glen Research) as recommended by the manufacturer on a 394 DNA/RNA Synthesizer (Applied Biosystems). 3′-BHQ-1 solid support (Biosearch Technologies) was used to introduce quenchers. ODNs were purified by denaturing HPLC with a Wave System (Transgenomic). ODN identity was verified by MALDI-TOF on a Voyager-DE mass spectrometer (Applied Biosystems). Observed masses were within 0.2% of the expected value (BHQ-1 easily fragmented, losing a mass of 297). Purity was ≥92% for all ODNs and was evaluated with capillary electrophoresis by using a capillary at 40°C filled with polyacrylamide and 7 M urea on a ^{3D}CE instrument (Agilent). ODNs without inosine, 7-deazaadenosine, 7-deazaxanthosine, 5-methylisocytidine, isoguanosine, or dye labels were purchased with anion exchange HPLC purification from Operon Biotechnologies. ODN **8**,

purified by anion exchange and then reverse-phase HPLC, was purchased from Biosearch Technologies. Extinction coefficients used to determine ODN concentrations at pH 7.0 were estimated as 90% of the sum of extinction coefficients at 260 nm for the component deoxynucleosides and dyes ($M^{-1}cm^{-1}$): 15,400, A; 11,700, G; 7,300, C; 8,800, T; 6,300, F; 4,600, J; 7,700, H; 9,400, Z; 8,440, X; 21,000, FAM; and 8,000, BHQ-1. Absorbance at 260 nm of N_1-H and N_3-H tautomers of J fortuitously appears to be nearly equivalent [16, 17].

Fluorescence Measurements

Experiments detecting fluorescence were performed by using either Mx4000 or Mx3000P instruments (Stratagene) with FAM filter sets. Cooling or heating ramps of samples in 96-well microplates were 0.5°C per 30 s over 10°C–90°C (Mx4000) or 25°C–90°C (Mx3000P); 75 μl sample volumes were used, and four data points were collected at each 0.5°C increment. Samples were measured in duplicate over one complete cooling and heating cycle. In stoichiometry experiments, ODN **1** at 125 nM was mixed with 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.25, 1.5, or 1.75 equivalents of **2**. Fluorescence of duplicate samples at each level was measured after a cooling ramp and then holding for 3 min at 25°C. SYBR Green I dye 10,000× stock solution (Molecular Probes) was diluted 1:99 with 0.045 M sodium citrate:0.45 M sodium chloride (pH 7.9). SYBR Green experiments were performed in 75 μl volumes containing 1 μl of the diluted SYBR Green solution with ODNs at 0.25 μM.

Absorbance Measurements

Experiments detecting absorbance were performed by using an 8453 spectrophotometer (Agilent) equipped with a Peltier thermostatted cell holder and an 89090A temperature controller. Thermal denaturation experiments were performed with 0.65 ml samples in black-wall microcells. Data were collected at 1 nm intervals and with a 0.5 s integration time, and volume correction was utilized for temperature changes. Cooling or heating ramps were 1°C per min over 20°C–80°C (for duplex **4**·**2**) or 20°C–85°C (for duplex **11**·**9**). Samples were measured over one or two complete cooling and heating cycles. Mixing curve samples of various mole fractions [15] of **4** and **2** were heated to 75°C for 3 min and then allowed to stand at room temperature for 24 hr. Mixing curve data were collected with the cell holder at 20°C.

CD Measurements

Samples contained 4 μM of each ODN strand in 0.65 ml in black-wall microcells. CD spectra were measured at 20°C and 75°C with an Aviv 62DS CD Spectrometer at 1 nm intervals and a 5 s averaging time.

Gel Analysis

Samples of **1** (18 pmol), **1**·**2** (18 pmol), **8** (9 pmol), and **8**·**9** (9 pmol) were made by adding the appropriate ODNs to 1 μl 0.45 M sodium citrate (pH 7.9) and diluting to 20 μl with water. The samples were heated at 75°C for 3 min, allowed to stand at room temperature for 90 min, and then kept at 4°C for 30 min. A 4% agarose E-gel (Invitrogen) was stored at 4°C for 30 min before electrophoresis. The top and bottom surfaces of the gel cassette were kept in contact with small, ice-filled plastic bags, and a current of 40 mA was applied for 2 min. Samples were loaded onto the gel, and a current of 20 mA was applied for 1 hr, keeping the gel cassette in contact with ice-filled plastic bags. The gel was imaged with a Gel Doc instrument (Bio-Rad) with Quantity One software.

Supplemental Data

Supplemental Data include seven figures and are available at http://www.chembiol.com/cgi/content/full/14/5/525/DC1/.

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REFERENCES

1. Crick, F.H.C. (1968). The origin of the genetic code. J. Mol. Biol. *38*, 367–379.
2. Wächtershäuser, G. (1988). An all-purine precursor of nucleic acids. Proc. Natl. Acad. Sci. USA *85*, 1134–1135.
3. Arnott, S., and Bond, P.J. (1973). Triple-stranded polynucleotide helix containing only purine bases. Science *181*, 68–69.
4. Williamson, J.R., Raghuraman, M.K., and Cech, T.R. (1989). Monovalent cation-induced structure of telomeric DNA: the G-quartet model. Cell *59*, 871–880.
5. Seela, F., Wei, C., and Melenewski, A. (1996). Isoguanine quartets formed by $d(T_4isoG_4T_4)$: tetraplex identification and stability. Nucleic Acids Res. *24*, 4940–4945.
6. Howard, F.B., Frazier, J., and Miles, H.T. (1975). Poly(8-bromoadenylic acid): synthesis and characterization of an all-syn polynucleotide. J. Biol. Chem. *250*, 3951–3959.
7. Howard, F.B., Hattori, M., Frazier, J., and Miles, H.T. (1977). A·I and A·G polynucleotide pairing. Controlling effect of amino group hydrogen bonds to solvent water. Biochemistry *16*, 4637–4646.
8. Kypr, J., Kejnovská, I., and Vorlíčková, M. (2003). DNA homoduplexes containing no pyrimidine nucleotide. Eur. Biophys. J. *32*, 154–158.
9. Rich, A. (1958). Formation of two- and three-stranded helical molecules by polyinosinic acid and polyadenylic acid. Nature *181*, 521–525.
10. Howard, F.B., and Miles, H.T. (1977). Interaction of poly(A) and poly(I). A reinvestigation. Biochemistry *16*, 4647–4650.
11. Geyer, C.R., Battersby, T.R., and Benner, S.A. (2003). Nucleobase pairing in expanded Watson-Crick-like genetic information systems. Structure *11*, 1485–1498.
12. Cherbuliez, E., and Bernhard, K. (1932). Recherches sur la graine de croton. I. Sur le crotonoside (2-oxy-6-amino-purine-d-riboside). Helv. Chim. Acta *15*, 464–471.
13. Fuhrman, F.A., Fuhrman, G.J., Nachman, R.J., and Mosher, H.S. (1981). Isoguanosine: isolation from an animal. Science *212*, 557–558.
14. Johansson, M.K., Fidder, H., Dick, D., and Cook, R.M. (2002). Intramolecular dimers: a new strategy to fluorescence quenching in dual-labeled oligonucleotide probes. J. Am. Chem. Soc. *124*, 6950–6956.
15. Gray, D.M., Hung, S.-H., and Johnson, K.H. (1995). Absorption and circular dichroism spectroscopy of nucleic acid duplexes and triplexes. Methods Enzymol. *246*, 19–34.
16. Sepiol, J., Kazimierczuk, Z., and Shugar, D. (1976). Tautomerism of isoguanosine and solvent-induced keto-enol equilibrium. Z. Naturforsch. [C] *31*, 361–370.
17. Seela, F., Wei, C., and Kazimierczuk, Z. (1995). Substituent reactivity and tautomerism of isoguanosine and related nucleosides. Helv. Chim. Acta *78*, 1843–1854.
18. Chen, X., Kierzek, R., and Turner, D.H. (2001). Stability and structure of RNA duplexes containing isoguanosine and isocytidine. J. Am. Chem. Soc. *123*, 1267–1274.
19. Horn, T., Chang, C.-A., and Collins, M.L. (1995). Hybridization properties of the 5-methyl-isocytidine/isoguanosine base pair in synthetic oligonucleotides. Tetrahedron Lett. *36*, 2033–2036.
20. Krishnamurthy, R., Pitsch, S., Minton, M., Miculka, C., Windhab, N., and Eschenmoser, A. (1996). Pyranosyl-RNA: base pairing

between homochiral oligonucleotide strands of opposite sense of chirality. Angew. Chem. Int. Ed. Engl. *35*, 1537–1541.

21. Groebke, K., Hunziker, J., Fraser, W., Peng, L., Diederichsen, U., Zimmermann, K., Holzner, A., Leumann, C., and Eschenmoser, A. (1998). Warum pentose- und nicht hexose-nucleinsäuren? Helv. Chim. Acta *81*, 375–474.
22. Roberts, C., Bandaru, R., and Switzer, C. (1997). Theoretical and experimental study of isoguanosine and isocytosine: base pairing in an expanded genetic system. J. Am. Chem. Soc. *119*, 4640–4649.
23. Ahle, J.D., Barr, S., Chin, A.M., and Battersby, T.R. (2005). Sequence determination of nucleic acids containing 5-methylisocytosine and isoguanine: identification and insight into polymerase replication of the non-natural nucleobases. Nucleic Acids Res. *33*, 3176–3184.
24. Gao, J., Liu, H., and Kool, E.T. (2005). Assembly of the complete eight-base artificial genetic helix, xDNA, and its interaction with the natural genetic system. Angew. Chem. Int. Ed. *44*, 3118–3122.
25. Sun, Z., Chen, D., Lan, T., and McLaughlin, L.W. (2002). Importance of minor groove functional groups for the stability of DNA duplexes. Biopolymers *65*, 211–217.
26. Thomas, K.A., Smith, G.M., Thomas, T.B., and Feldmann, R.J. (1982). Electronic distributions within protein phenylalanine aromatic rings are reflected by the three-dimensional oxygen environments. Proc. Natl. Acad. Sci. USA *79*, 4843–4847.
27. Orgel, L.E. (2004). Prebiotic chemistry and the origin of the RNA world. Crit. Rev. Biochem. Mol. Biol. *39*, 99–123.
28. Orgel, L.E. (1968). Evolution of the genetic apparatus. J. Mol. Biol. *38*, 381–393.
29. Zubay, G., and Mui, T. (2001). Prebiotic synthesis of nucleotides. Orig. Life Evol. Biosph. *31*, 87–102.
30. Ricardo, A., Carrigan, M.A., Olcott, A.N., and Benner, S.A. (2004). Borate minerals stabilize ribose. Science *303*, 196.
31. Schneeberger, C., Speiser, P., Kury, F., and Zeillinger, R. (1995). Quantitative detection of reverse transcriptase-PCR products by means of a novel and sensitive DNA stain. PCR Methods Appl. *4*, 234–238.
32. Eschenmoser, A. (1999). Chemical etiology of nucleic acid structure. Science *284*, 2118–2124.
33. Venables, W.N., and Ripley, B.D. (2002). Non-linear and smooth regression. In Modern Applied Statistics with S (New York: Springer), pp. 211–250.