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DNA homoduplexes containing no pyrimidine nucleotide

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Abstract We show using polyacrylamide gel electrophoresis that guanine + adenine repeat strands of DNA associate into homoduplexes at neutral pH and moderate ionic strength. The homoduplexes melt in a cooperative way like the Watson-Crick duplex, although they contain no Watson-Crick base pair. Guanine is absolutely needed for the homoduplex formation and the homoduplex stability increases with the guanine content of the repeat. The present results have implications for the nature of the first replicators, as well as regarding forces stabilizing the duplexes of DNA.

Keywords Adenine · Base pairing · Circular dichroism spectroscopy · DNA homoduplexes · Guanine

Introduction

The genetic information of all known free organisms is deposited in molecules of double-stranded DNA. The double strandedness is required to ensure replication whose fidelity is based on pairing of the complementary purine and pyrimidine bases (Watson and Crick 1953). However, cytosine is not sufficiently stable under prebiotic conditions (Shapiro 1999) so the GC base pair may not have been used in the first genetic material unless life arose in an unlikely short period of time (Levy and Miller 1998). This makes interesting the possibility of the existence and stability of all-purine duplexes of DNA (Wächtershäuser 1988). In fact, the duplexes should be homoduplexes because the simultaneous occurrence of different complementary strands is much less probable.

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Guanine strands of DNA, even dinucleotides, are known to associate strongly to form tetraplexes and much larger aggregates that are based on guanine tetrads (reviewed in Guschlbauer et al. 1990; Shafer 1998; Hardin et al. 2001; Keniry 2001). However, the stability of the guanine tetraplexes is so high that they are unsuitable for replication (Woodford et al. 1994; Usdin and Woodford 1995; Weitzmann et al. 1996, 1997; Han et al. 1999). Hence guanine strands could hardly fulfill the role of the first replicators. On the other hand, adenine strands associate into homoduplexes only at very acid pH values (Rich et al. 1961; Finch and Klug 1969), so that they also are not appropriate candidates for the first replicators. Both of these unfavorable properties are eliminated in DNA strands containing both guanine and adenine. For example, $d(GA)_{10}$ strands homodimerize at physiological pH and salt concentrations and the homoduplex was found to melt with a similar cooperativity as the Watson-Crick homoduplex of $d(TA)_{10}$ (Vorlíčková et al. 1999). Remarkably, guanine and adenine are the first members of the series describing adsorption of organic molecules onto the surfaces of inorganic solids that has long been considered a process relevant to the origin of life (Sowerby et al. 2001). Here we examine a series of 20-mer or 21-mer guanine + adenine repeat strands of DNA other than $d(GA)_{10}$ to see whether they homodimerize as well and how their homodimerization depends on the repeat primary structure and its guanine content.

Materials and methods

The DNA oligonucleotides $d(GGGA)_5$ (extinction coefficient 12,070 M^{-1} cm^{-1} at 255 nm and 90 °C), $d(GGAA)_5$ (12, 150 M^{-1} cm^{-1} at 256 nm and 90 °C), $d(GA)_{10}$ (10,820 M^{-1} cm^{-1} at 255 nm and 25 °C), $d(GAAA)_5$ (10,750 M^{-1} cm^{-1} at 257 nm and 25 °C) and $d(A)_{20}$ (10,620 M^{-1} cm^{-1} at 258 nm and 25 °C) were synthesized and purified by VBC Genomics Bioscience (Vienna, Austria). The oligonucleotides $d(GGA)_7$ (extinction coefficient 12,280 M^{-1} cm^{-1} at 255 nm and 90 °C) and $d(GAA)_7$ (10,850 M^{-1} cm^{-1} at 255 nm and 25 °C) were bought from the Laboratory of Plant Molecular Physiology, Faculty of Science, Masaryk University

(Brno, Czech Republic). The lyophilized oligonucleotides were dissolved in 1 mM sodium phosphate and 0.3 mM EDTA, pH 7.4, to give a stock solution concentration of ~100 OD/mL.

The DNA concentrations were determined from their absorption measured in the above buffer on a Unicam 5625 UV/Vis spectrometer. UV absorption and CD spectra of some (especially the G-rich) oligonucleotides differed before and after thermal denaturation because they contained aggregates formed in the concentrated stock solutions. To ensure reproducibility, all of the samples were denatured before starting any measurements.

CD spectra were measured on the Jobin-Yvon Mark IV and VI dichrographs in 0.1 cm (unless stated otherwise) pathlength Hellma cells placed in a thermostatted holder. The dichrographs were calibrated with isoandrosterone. The DNA concentration was chosen to give absorption of ~0.8 at the absorption maximum because this gives the optimum signal-to-noise ratio. $\Delta\epsilon$ was expressed in units of M^{-1} cm^{-1}, the molarity (M) being related to the number of nucleoside residues in the DNA samples. LiCl was added directly to the cells. The salt and DNA concentrations were corrected for the sample volume increase. The pH values were measured using a Sentron Titan pH meter and the Sentron Red-Line electrode. The electrode measures pH in volumes as low as 3 μL.

The non-denaturing polyacrylamide gel electrophoresis was carried out in thermostatted SE-600 (Hoefer) submarine apparatus. The gels were 16% (29:1 monomer/bis ratio) and had 14×16×0.1 cm^3 dimensions. The gels were run for 20–24 h at 60 V in 4 L of the buffer circulating at 2 °C with the aid of a Huber cryostat. DNA was 0.8 mM (in nucleoside residues) in the electrophoresed samples. The gels were stained with Stains-All (Sigma) and scanned by Personal Densitometer SI, model 375-A (Molecular Dynamics). The guanine-rich samples were exposed to 85 °C for 5 min before electrophoresis. Their buffer (1 mM Na-phosphate, 0.3 mM EDTA, pH 7.6) was adjusted using 10× concentrated electrophoretic buffer to make the sample buffer similar to the electrophoresis buffer.

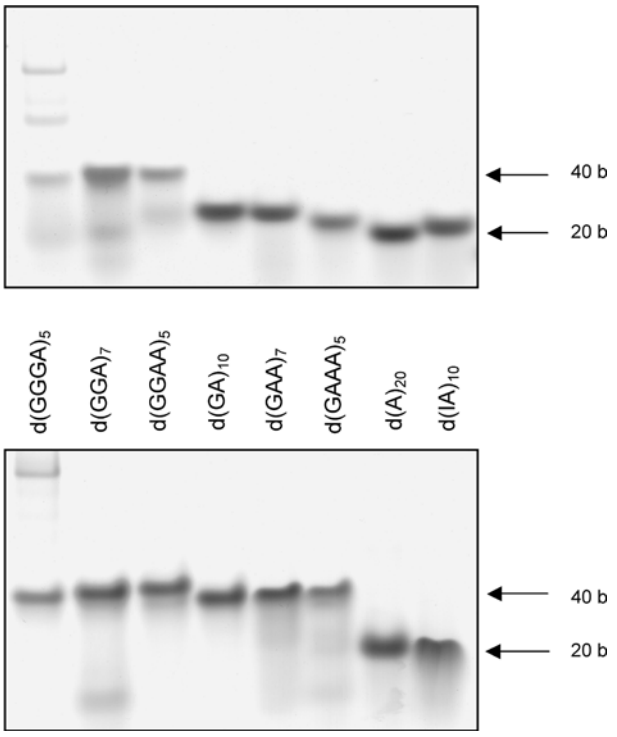

Fig. 1 Polyacrylamide gels of $d(GGGA)_5$, $d(GGA)_7$, $d(GGAA)_5$, $d(GA)_{10}$, $d(GAA)_7$, $d(GAAA)_5$, $d(A)_{20}$ and $d(IA)_{10}$ (a single-stranded standard; dI stands for deoxyinosine, i.e. deoxyguanosine lacking the amino group) run at (*top*) low salt (1 mM Na-phosphate, 0.3 mM EDTA, pH 7.6, temperature 2 °C) and (*bottom*) moderate salt (10 mM Na-phosphate, 0.15 M LiCl, 0.3 mM EDTA, pH 7.1, temperature 2 °C) conditions

Results

Figure 1 shows a low-salt (1 mM sodium phosphate, 0.3 mM EDTA, pH 7.6, temperature 2 °C) polyacrylamide gel of a series of adenine + guanine repeat strands of DNA. Under these conditions, $d(A)_{20}$, $d(GAAA)_5$, $d(GAA)_7$ and $d(GA)_{10}$ migrated as single strands, whereas the major parts of $d(GGAA)_5$, $d(GGA)_7$ and $d(GGGA)_5$ were homoduplexes. The sample of $d(GGGA)_5$ also contained a tetraplex and a heavier discrete species, presumably composed of eight $d(GGGA)_5$ strands, that was resistant to exposure to 85 °C for 5 min. Figure 1 also shows a similar gel run at a moderate LiCl concentration (10 mM sodium phosphate, 0.3 mM EDTA, 0.15 M LiCl, pH 7.1, temperature 2 °C). The increased ionic strength caused only $d(A)_{20}$ to remained single stranded, whereas $d(GAAA)_5$, $d(GAA)_7$, $d(GA)_{10}$, $d(GGAA)_5$, $d(GGA)_7$ and $d(GGGA)_5$ were dominantly homoduplexes. The presence of LiCl eliminated the tetraplex of $d(GGGA)_5$ and a major part of its eight-stranded aggregate (Fig. 1).

The $d(GA)_{10}$ 20-mer was denatured at low salt and provided a weak CD spectrum (Fig. 2). Addition of LiCl caused dramatic changes in the CD spectrum. The oligonucleotide CD was substantially enhanced to indicate base ordering. The CD of $d(GA)_{10}$ especially was increased at 265 nm and at 245 nm. The amplitudes of these bands finally several times exceeded the corresponding values observed for the canonical B-form. The CD spectral changes were essentially complete at 0.5 M LiCl. Further additions of LiCl negligibly changed the CD spectrum. However, the CD still slightly increased upon temperature lowering (Fig. 2).

Figure 3 shows the limiting LiCl-induced changes in the CD spectra of the remaining six 20-mers and 21-mers explored in this work. The CD spectrum of $d(A)_{20}$ was small and insensitive to LiCl, which was consistent with the electrophoretic data indicating that $d(A)_{20}$ was denatured under all conditions tested in this work. Though it only contained 25% of G, $d(GAAA)_5$ was very sensitive to the concentration of LiCl. It showed similar CD spectral changes as $d(GA)_{10}$ and $d(GAA)_7$. The other couple of the present DNA fragments, i.e. $d(GGAA)_5$ and $d(GGA)_7$, also qualitatively showed a similar CD spectral behavior. The spectral changes were rather suppressed with $d(GGAA)_5$ to indicate that the primary structure of the repeat was also a factor contributing to the homoduplex formation with the present guanine + adenine repeat DNA fragments. The 20-mer $d(GGGA)_5$ provided the CD spectrum of the homoduplex of $d(GA)_{10}$ in the absence of LiCl, whose additions only caused minor changes. LiCl eliminated tetraplexes formed by $d(GGGA)_5$ and $d(GGA)_7$, especially in the presence of KCl (not shown).

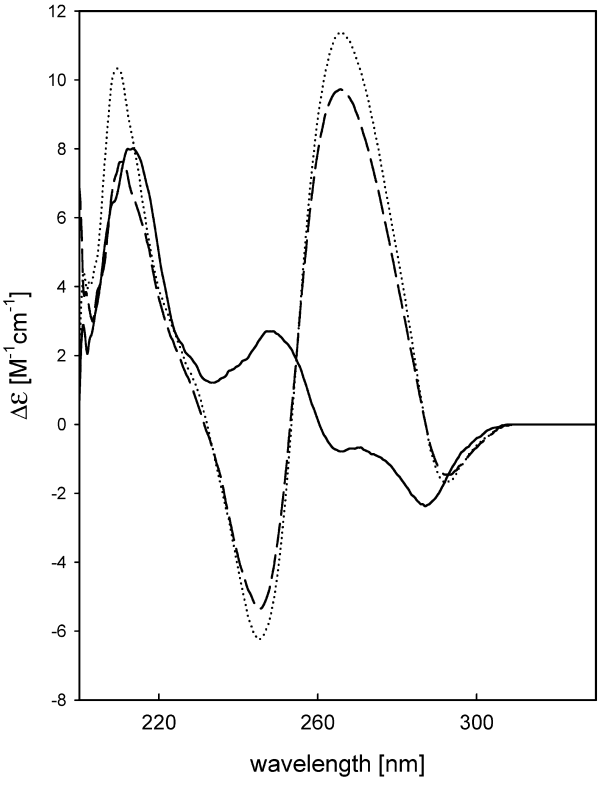

Fig. 2 CD spectra of denatured $d(GA)_{10}$ in 10 mM Na-phosphate, 0.3 mM EDTA, measured at 22 °C (*solid curve*), the homoduplex of $d(GA)_{10}$ induced by the addition of LiCl up to 0.5 M concentration (*dashed curve*), and the same sample after temperature lowering from 22 °C to 0 °C (*dotted curve*)

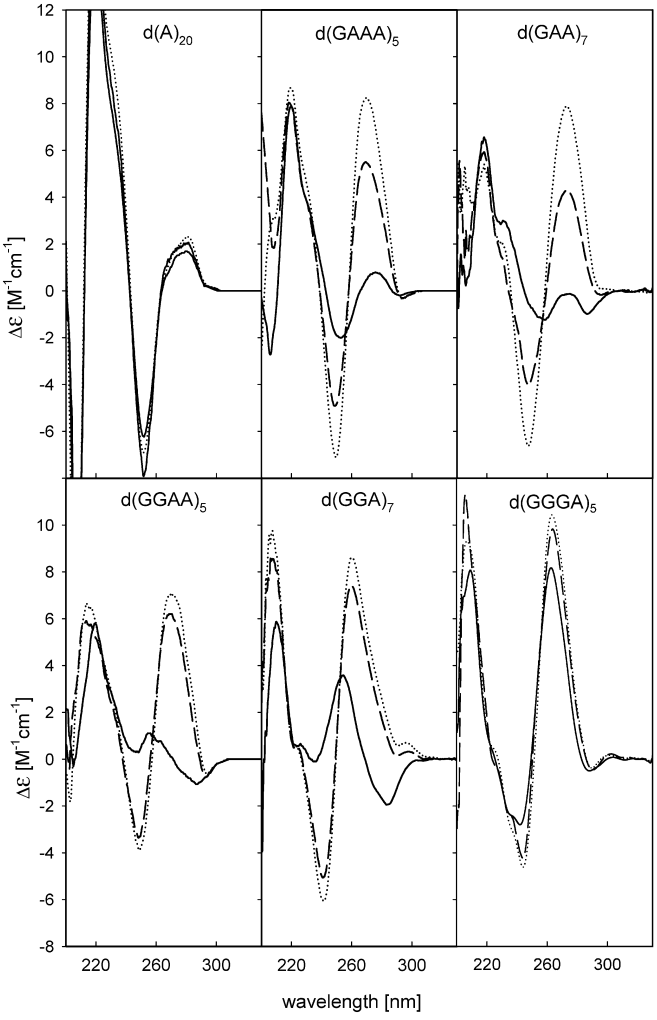

Fig. 3 CD spectra as in Fig. 2, but for $d(A)_{20}$, $d(GAAA)_5$, $d(GAA)_5$, $d(GGAA)_5$, $d(GGA)_7$ and $d(GGGA)_5$, measured in 10 mM Na-phosphate, 0.3 mM EDTA, pH 7.1, temperature 22 °C (*solid curves*), in 10 mM Na-phosphate, 0.3 mM EDTA, and the LiCl concentrations specified below, pH 7.1, at 22 °C (*dashed curves*), and in the same buffer and LiCl concentrations, but at 0 °C (*dotted curves*). The concentrations of LiCl were 0.3 M with $d(A)_{20}$, 0.6 M with $d(GAAA)_5$, 0.5 M with $d(GAA)_7$, 0.5 M with $d(GGAA)_5$, 0.25 M with $d(GGA)_7$ and 0.3 M with $d(GGGA)_5$

The ordered LiCl-stabilized conformers of DNA melted in a cooperative way with the increasing temperature. The cooperativity is a very important, though poorly understood, aspect of biological functioning of DNA. We measured the temperature dependences of the CD spectra of the seven present DNA fragments in the presence of 0.15 M LiCl where homoduplexes predominated in the samples (Fig. 1). Figure 4 shows temperature dependences of the positive CD band that exhibited the largest changes. First of all, Fig. 4 demonstrates that $\Delta\epsilon$ of $d(A)_{20}$ changed little with temperature and that it lacked the sigmoidal shape characteristic for cooperative phenomena. In contrast, the remaining six oligonucleotides present showed the sigmoidal shapes, indicating that their homoduplexes arose and denatured in a cooperative way. The midpoints of the curves mostly shifted towards higher temperatures with the increasing guanine content in the repeat. There was, however, no significant difference between $d(GAA)_7$ and $d(GAAA)_5$, whereas $d(GA)_{10}$ and $d(GGAA)_5$ had the midpoints rather different though their guanine content was the same. Hence the primary structure was another factor influencing the homoduplex thermostability, though less important than the guanine content.

Discussion

Numerous recent experiments demonstrate that hydrogen bonding between the complementary bases is necessary neither for DNA duplex stability (Wu et al. 2000), its replication (Guckian et al. 1998; Morales and Kool 1998; Morales and Kool 2000; Dzantiev et al. 2001; Tae et al. 2001) nor transcription (Ohtsuki et al. 2001), because various couples of bases lacking the hydrogen bonding capacity stabilize the DNA duplex as well and are even replicated or transcribed by the native polymerases. This opens the question of the first replicators in the evolutionary pathway leading to the current DNA.

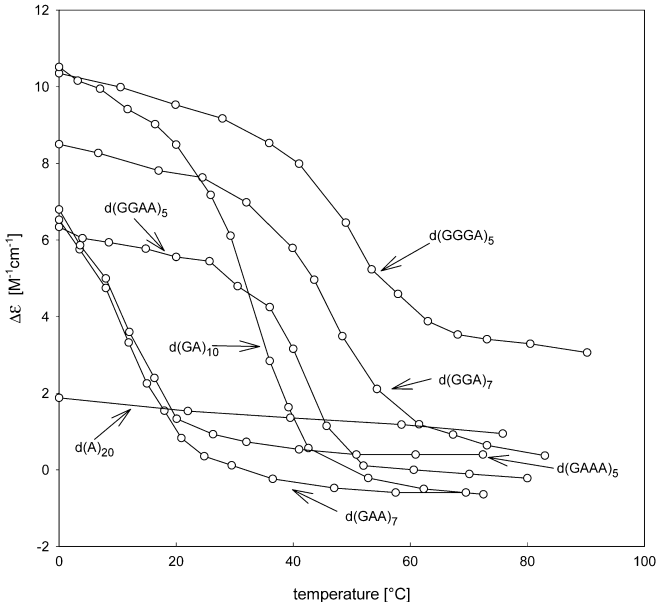

Fig. 4 Temperature dependences of the amplitudes of the positive CD bands in the vicinity of 270 nm for $d(GGGA)_5$, $d(GGA)_7$, $d(GGAA)_5$, $d(GA)_{10}$, $d(GAA)_7$, $d(GAAA)_5$ and $d(A)_{20}$. All of the dependences were measured in 10 mM Na-phosphate, 0.15 M LiCl, 0.3 mM EDTA, pH 7.1

RNA preceded DNA in evolution (Gilbert 1986), but even RNA was hardly the first replicator because of the low stability of its backbone (Joyce et al. 1987; Larralde et al. 1995). A peptide nucleic acid (PNA) is a much more promising candidate for the first replicator (Orgel 1998; Nielsen 1999). It is also highly improbable that the first replicators contained four different bases (Shapiro 1995). Rather, they contained a single base or two very similar bases because the simultaneous occurrence of four bases at the same time and place is incomparably less probable. Finally, the bases present in the first replicators were of purine rather than pyrimidine origin (Wächtershäuser 1988). Taking all of the above together, the first replicators probably were based on guanine or a similar molecule having strong self-association tendency (Gellert et al. 1962; Ralph et al. 1962). However, the self-association tendency of guanine is too strong because it inhibits replication (Woodford et al. 1994; Usdin and Woodford 1995; Weitzmann et al. 1996, 1997; Han et al. 1999). So guanine should be combined with another base, diluting its strong self-associations in the first replicators. In this direction, adenine is a possible candidate as it belongs among the oligomerization products of prebiotic reactions involving HCN (Shapiro 1995).

Guanine + adenine strands of DNA have been known to self-associate for more than 20 years (Lee et al. 1980). However, they were originally thought to be tetrastranded (Lee et al. 1980; Lee 1990), which is not the best number of strands for replication. It was only later shown that the alternating guanine-adenine repeat strands of DNA associated into homoduplexes (Vorlíčková et al. 1999), both parallel (Rippe et al. 1992) and antiparallel (Casasnovas et al. 1993; Huertas et al. 1993). We showed (Vorlíčková et al. 1999; Kypr and Vorlíčková 2001) that the homodimerization of the alternating guanine-adenine repeat strands of DNA resembled the leucine zipper, a motif frequently ensuring dimerization of proteins. This enhances interest into the possibility of a common evolutionary origin of DNA and proteins and the role of PNA or similar molecules as their joint predecessor (Kypr and Vorlíčková 2001, 2002).

Here we have undertaken a study of the role of the repeat primary structure and its guanine content in the homodimerization of guanine + adenine repeat strands of DNA. The first conclusion following from the present study is that the homodimerization is not a specific property of the alternating $d(GA)_n$ repeat because other repeats homodimerize as well. The presence of guanine is absolutely necessary for the repeat to homodimerize because $d(A)_{20}$ did not homodimerize under any conditions tested in this work. However, 25% guanine content in the repeat was sufficient for the $d(GAAA)_5$ strand to homodimerize. Higher than 50% guanine content caused $d(GGA)_7$ and $d(GGGA)_5$ to associate into tetraplexes in the stock solutions, where their concentrations were very high. They also generated tetraplexes in the presence of KCl (not shown), but they were homoduplexes in the presence of LiCl. Melting points of the tetraplexes of $d(GGGA)_5$ and $d(GGA)_7$ were, respectively, 32 °C and 19 °C higher in 0.15 KCl compared to the homoduplexes stable in 0.15 M LiCl. The remaining four guanine + adenine repeat strands of DNA analyzed in this work had a guanine content of 50% or less. They associated into tetraplexes neither in the stock solution nor in the presence of KCl, and they generated homoduplexes as soon as the ionic strength was sufficient to compensate for the interstrand phosphate-phosphate repulsion. The stability of the homoduplexes decreased with the increasing number of adenines in the repeat.

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References

Casasnovas JM, Huertas D, Ortiz-Lombardía M, Kypr J, Azorín F (1993) Structural polymorphism of $d(GA.TC)_n$ DNA sequences: intramolecular and intermolecular associations of the individual strands. J Mol Biol 233:671–681

Dzantiev L, Alekseyev YO, Morales JC, Kool ET, Romano LJ (2001) Significance of nucleobase shape complementarity and hydrogen bonding in the formation and stability of the closed polymerase-DNA complex. Biochemistry 40:3215–3221

Finch JT, Klug A (1969) Two double helical forms of polyriboadenylic acid and the pH-dependent transition between them. J Mol Biol 46:597–598

Gellert BM, Lipsett MN, Davies DR (1962) Helix formation by guanylic acid. Proc Natl Acad Sci USA 48:2013–2019

Gilbert W (1986) The RNA world. Nature 319:618

Guckian KM, Morales JC, Kool ET (1998) Structure and base pairing properties of a replicable nonpolar isostere for deoxyadenosine. J Org Chem 63:9652–9656

Guschlbauer W, Chantot J-F, Thiele D (1990) Four-stranded nucleic acid structures 25 years later: from guanosine gels to telomer DNA. J Biomol Struct Dyn 8:491–511

Han H, Hurley LH, Salazar M (1999) A DNA polymerase stop assay for G-quadruplex interactive compounds. Nucleic Acids Res 27:537–542

Hardin CC, Perry AG, White K (2001) Thermodynamic and kinetic characterization of the dissociation and assembly of quadruplex nucleic acids. Biopolymers 56:147–194

Huertas D, Bellsolell L, Casasnovas JM, Coll M, Azorín F (1993) Alternating $d(GA)_n$ DNA sequences form antiparallel stranded homoduplexes stabilized by the formation of G.A base pairs. EMBO J 12:4029–4038

Joyce GF, Schwartz AW, Miller SL, Orgel LE (1987) The case for an ancestral genetic system involving simple analogues of the nucleotides. Proc Natl Acad Sci USA 84:4398–4402

Keniry MA (2001) Quadruplex structures in nucleic acids. Biopolymers 56:123–146

Kypr J, Vorlickova M (2001) Dimethylsulphoxide-stabilized conformer of guanine-adenine repeat strand of DNA. Biopolymers (Biospectroscopy) 62:81–84

Kypr J, Vorlickova M (2002) Circular dichroism spectroscopy reveals invariant conformation of guanine runs in DNA. Biopolymers (Biospectroscopy) 67:275–277

Larralde R, Robertson MP, Miller SL (1995) Rates of decomposition of ribose and other sugars: implications for chemical evolution. Proc Natl Acad Sci USA 92:8158–8160

Lee JS (1990) The stability of polypurine tetraplexes in the presence of mono- and divalent cations. Nucleic Acids Res 18:6057–6060

Lee JS, Evans DH, Morgan AR (1980) Polypurine DNAs and RNAs form secondary structures which may be tetra-stranded. Nucleic Acids Res 8:4305–4320

Levy M, Miller SL (1998) The stability of the RNA bases: implications for the origin of life. Biochemistry 95:7933–7938

Morales JC, Kool ET (1998) Efficient replication between non-hydrogen-bonded nucleoside shape analogs. Nat Struct Biol 5:950–954

Morales JC, Kool ET (2000) Functional hydrogen-bonding map of the minor groove binding tracks of six DNA polymerases. Biochemistry 39:12979–12988

Nielsen PE (1999) Peptide nucleic acid. A molecule with two identities. Acc Chem Res 32:624–630

Ohtsuki T, Kimoto M, Ishikawa M, Mitsui T, Hirao I, Yokoyama S (2001) Unnatural base pairs for specific transcription. Proc Natl Acad Sci USA 98:4922–4925

Orgel LE (1998) The origin of life: a review of facts and speculations. Trends Biochem Sci 23:491–495

Ralph RK, Connors WJ, Khorana HG (1962) Secondary structure and aggregation in deoxyguanosine oligonucleotides. J Am Chem Soc 84:2265–2266

Rich A, Davies DR, Crick FHC, Watson JD (1961) The molecular structure of polyadenylic acid. J Mol Biol 3:71–86

Rippe K, Fritsch V, Westhof E, Jovin TM (1992) Alternating d(G-A) sequences form a parallel-stranded DNA homoduplex. EMBO J 11:3777–3786

Shafer RH (1998) Stability and structure of model DNA triplexes and quadruplexes and their interactions with small ligands. Prog Nucleic Acid Res Mol Biol 59:55–94

Shapiro R (1995) The prebiotic role of adenine: a critical analysis. Origins Life Evol Biosphere 25:83–98

Shapiro R (1999) Prebiotic cytosine synthesis: a critical analysis and implications for the origin of life. Proc Natl Acad Sci USA 96:4396–4401

Sowerby SJ, Cohn CA, Heckl WM, Holm NG (2001) Differential adsorption of nucleic acid bases: relevance to the origin of life. Proc Natl Acad Sci USA 98:820–822

Tae EL, Wu Y, Xia G, Schultz PG, Romesberg FE (2001) Efforts toward expansion of the genetic alphabet: replication of DNA with three base pairs. J Am Chem Soc 123:7439–7440

Usdin K, Woodford KJ (1995) CGG repeats associated with DNA instability and chromosome fragility form structures that block DNA synthesis in vitro. Nucleic Acids Res 23:4202–4209

Vorlíčková M, Kejnovská I, Kovanda J, Kypr J (1999) Dimerization of the guanine-adenine repeat strands of DNA. Nucleic Acids Res 27:581–586

Watson JD, Crick FH (1953) Genetical implications of the structure of deoxyribonucleic acid. Nature 171:964–967

Wächtershäuser G (1988) An all-purine precursor of nucleic acids. Proc Natl Acad Sci USA 85:1134–1135

Weitzmann MN, Woodford KJ, Usdin K (1996) The development and use of a DNA polymerase arrest assay for the evaluation of parameters affecting intrastrand tetraplex formation. J Biol Chem 271:20958–20964

Weitzmann MN, Woodford KJ, Usdin K (1997) DNA secondary structures and the evolution of hypervariable tandem arrays. J Biol Chem 272:9517–9523

Woodford KJ, Howell RM, Usdin K (1994) A novel K^+-dependent DNA synthesis arrest site in a commonly occurring sequence motif in eukaryotes. J Biol Chem 269:27029–27035

Wu Y, Ogawa AK, Berger M, McMinn DL, Schultz PG, Romesberg FE (2000) Efforts toward expansion of the genetic alphabet: optimization of interbase hydrophobic interactions. J Am Chem Soc 122:7621–7632