A mobility shift assay, using native PAGE (Figure 1), strengthens the picture. As the figure shows, strand association at low salt is seen with the 3′,5″-linked **13/14** combination by retardation of labelled **14**, but no association can be detected for 2′,5″-mixed sequences **11/12**, for 2′,5″ homopolymers **15/16**, or for 2′,5″-**11** with 3′,5″-**14**.

Switzer reports[7] T_m (lower than for normal DNA) evidence for association of $(3'\text{-deoxyA})_{12}$ with $(3'\text{-deoxyU})_{12}$ at high salt. Our T_m studies confirm some association in our related **16/17**, but *only* at high salt. Recent studies on 2′,5″-linked RNA 6–10-mers also demonstrate weak strand association at high salt concentrations.[8] However, it is apparent from all this that, if duplex formation occurs with 3′-deoxy isomeric DNA, it is at best weaker than that for normal DNA and is seen only with high salt. This lack of strong association, especially under normal cellular conditions, may be the selective disadvantage that eliminated 2′,5″-linked nucleic acids from a role as genetic material.

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Self-Association of 2′,5′-Linked Deoxynucleotides: Meta-DNA

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Both 3′,5′- and 2′,5′-linked nucleotides occur naturally,[1] but only nucleotides with the former linkage encode genetic information. In spite of this fact, activated mononucleotides generally give 2′,5′-linked nucleotides during abiotic oligomerization,[2,3] suggestive of a prebiotic bias toward this connectivity. Thus, the question presents itself as to whether the 2′,5′-internucleotide linkage arose first at the time of natural history during which chemical evolution is postulated and was later superseded by the 3′,5′-linkage during prebiotic or early biotic evolution. Such a scenario could be plausibly integrated into the currently held view that RNA may have served a central role in the origins of life.[4]

A successful genetic material must be capable of directing its reproduction. 3′,5′-Linked nucleic acids accomplish this through self-association by way of Watson–Crick base pairing. Whether 2′,5′-linked nucleotides can similarly self-associate has been the object of various hypotheses. These have been based upon studies of dinucleotides,[5] or theoretical calculations,[6] and lead to conflicting predictions. We report the synthesis of single-stranded DNA segments containing only "nongenomic" 2′,5′-internucleotide linkages, for which we propose the name "meta-DNA" (Figure 1), and find that they do self-associate.

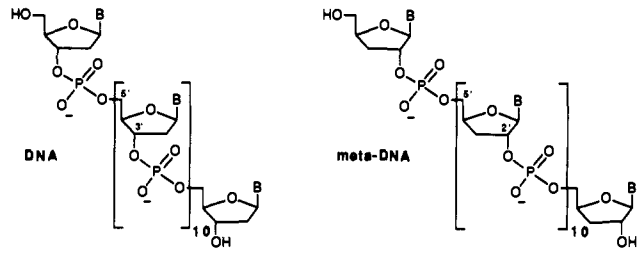

Figure 1.

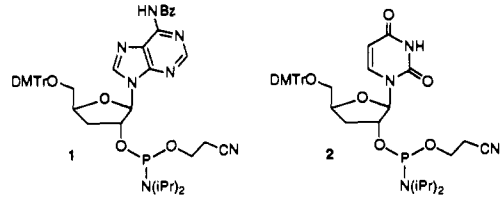

Figure 2.

We have synthesized three meta-DNA strands: a mutually complementary pair of 3′-deoxydodecanucleotides, meta-dA_{12} and meta-dU_{12}, and one that was chosen to be self-complementary, meta-$d(AU)_6$.[7,8] The starting materials were phosphoramidites **1** and **2** (Figure 2), which were prepared by direct extensions of standard methods.[9] After deprotection, the oligomers were purified by high-performance liquid chromatography (HPLC),[10] and their composition was verified by laser desorption mass spectrometry and also by digestion to nucleoside monomers (snake venom phosphodiesterase in the presence of bacterial alkaline phosphatase),[11] followed by HPLC comparison with authentic monomer samples.

Self-association of meta-DNA dodecamers was assayed by examining their ultraviolet (UV) absorbance profiles versus temperature. Such profiles are well known to reveal hyperchromic effects due to the decrease in base-stacking interactions that occurs when genomic 3′,5′-linked DNA double helices dissociate to single strands.[12] These profiles and their first derivatives for the various dodecamers are reproduced in Figures 3 and 4.

As can be seen in Figure 3A, a mixture of meta-dA_{12} and meta-dU_{12} exhibits a dramatic hyperchromic change in UV absorbance between 5 and 25 °C, amounting to approximately 60% of the analogous effect observed with a mixture of genomic dA_{12} and dT_{12}. This change is indicative of the dissociation of a meta-DNA complex. Analysis of the derivative curves for the absorbance profiles versus temperature (Figure 3B) allows the

(1) The 2′,5′-linkage is found in oligoadenylate messengers involved in the interferon-induced response to viral infection in eukaryotes, see: Adams, R. L. P.; Knowler, J. T.; Leader, D. P. *The Biochemistry of the Nucleic Acids*; Chapman and Hall: New York, 1986; pp 441–442.

(2) For oligomerizations in the absence of oligonucleotide templates, a situation where the bias toward a 2′,5′-linkage is generally acute and is obviously lacking any influence of an added template, see: (a) Lohrmann, R.; Orgel, L. E. *Tetrahedron* **1978**, *34*, 853. (b) Sawai, H. *J. Mol. Evol.* **1988**, *27*, 181.

(3) For reviews of oligomerizations in the presence of 3′,5′-linked RNA templates, see: (a) Joyce, G. F. *Cold Spring Harbor, Symposia on Quantitative Biology*; Cold Spring Harbor: New York, 1987; Vol. LII, pp 41–52. (b) Orgel, L. E. *J. Theor. Biol.* **1986**, *123*, 127.

(4) Reviewed in the following: Joyce, G. F. *Nature* **1989**, *338*, 217 and ref 3b.

(5) (a) Parthasarathy, R.; Malik, M.; Fridey, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 7292. (b) Dhingra, M. M.; Sarma, R. H. *Nature* **1978**, *272*, 798.

(6) (a) Srinivasan, A. R.; Olson, W. K. *Nucleic Acids Res.* **1986**, *14*, 5461. (b) Anukanth, A.; Ponnuswamy, P. K. *Biopolymers* **1986**, *25*, 729.

(7) Synthesis was performed on a controlled-pore glass support derivatized with the appropriate 5′-(dimethoxytrityl)-3′-deoxynucleoside using an Applied Biosystems 391 EP DNA synthesizer. For the derivatization procedure, see: Atkinson, T.; Smith, M. *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1985; pp 47–49. DNA synthesis was performed on a 1-μmol scale.

(8) The following corresponding genomic 2′-deoxydodecanucleotides were also prepared as references in this work: dA_{12}, dT_{12}, and $d(AT)_6$.

(9) Phosphoramidites **1** and **2** were synthesized from 3′-dA and 5′-(dimethoxytrityl)-3′-dU, respectively, using the same procedures reported for the corresponding 2′-deoxynucleosides: Atkinson, T.; Smith, M. *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford 1985; pp 39–45. 3′-dA synthesis: Hansske, F.; Robins, M. J. *Tetrahedron Lett.* **1985**, *26*, 4295. 5′-(Dimethoxytrityl)-3′-dU synthesis: Ogilvie, K. K.; Hakimelahi, G. H.; Proba, Z. A.; Usman, N. *Tetrahedron Lett.* **1983**, *24*, 865. 3′-dA and 3′-dU (prepared by detritylation of its parent[10]) were spectroscopically identical with authentic samples purchased from Sigma Chemical Co.

(10) Deprotection (30% ammonium hydroxide in water, 55 °C, 15 h) and then reversed-phase HPLC purification was followed by detritylation (80% aqueous acetic acid).

(11) (a) Herdewijn, P.; Charubala, R.; Pfleiderer, W. *Helv. Chim. Acta* **1989**, *72*, 1729. (b) Eritja, R.; et al. *Nucleic Acids Res.* **1986**, *14*, 8135.

(12) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984; pp 143–146.

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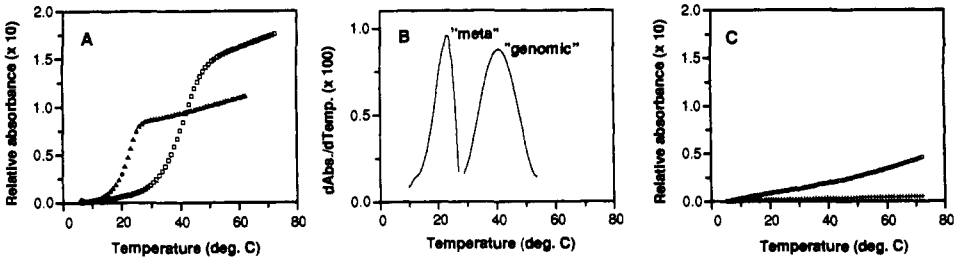

Figure 3. (A) UV absorbance profiles versus temperature for meta-dA_{12}/meta-dU_{12} (△) and genomic dA_{12}/dT_{12} (□). Measurements were determined by monitoring absorbance at 260 nm. The sample buffer in all cases contained 1 M NaCl, 10 mM sodium phosphate, and 0.1 mM EDTA in H_2O at pH 7. Oligonucleotide concentration was 2.5 μM in each strand. All results are derived from duplicate experiments. Profiles have been normalized to zero absorbance at 5 °C. (B) First derivatives of the UV absorbance profiles versus temperature for meta-dA_{12}/meta-dU_{12} and genomic dA_{12}/dT_{12}. (C) UV absorbance profiles versus temperature for meta-dA_{12} (○) and meta-dU_{12} (+). See part A above for experimental conditions.

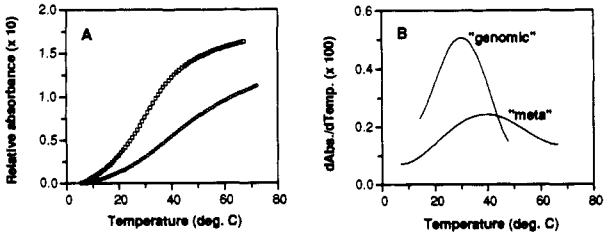

Figure 4. (A) UV absorbance profiles versus temperature for meta-d$(AU)_6$ (○) and genomic d$(AT)_6$ (□). The oligonucleotide concentration was 5 μM; see the caption of Figure 3A for additional experimental details. Profiles have been normalized to zero absorbance at 5 °C. (B) First derivatives of the UV absorbance profiles versus temperature for meta-d$(AU)_6$ and genomic d$(AT)_6$.

determination of the melting temperatures (T_m's) of the meta and genomic mutually complementary dodecamers as 22.8 and 40.8 °C, respectively. It is also apparent from the derivative curves that the denaturation of the meta dodecamers is more cooperative than that of the genomic ones. Somewhat different behavior was observed for the self-complementary meta-d$(AU)_6$ (T_m = 39.3 °C) and genomic d$(AT)_6$ (T_m = 29.9 °C) (Figure 4). Here, in contrast to the previous case, the denaturation of the self-complementary meta dodecamer is *less* cooperative and occurs with a *higher* T_m value than that of the genomic one.

To probe the nature of the structure in the first example involving meta-dA_{12} and meta-dU_{12}, controls were performed where absorbance profiles versus temperature were determined for pure meta-dA_{12} and pure meta-dU_{12}, separately (Figure 3C). These profiles rule out the possibilities of purinic self-association[13] or pyrimidinic self-association,[14] leading to the profile that resulted from the admixture of meta-dA_{12} and meta-dU_{12} (Figure 3A), and permit the observation that meta-dA_{12} and meta-dU_{12} complex with one another in a manner consistent with classical, Watson–Crick base pairing.[15]

Comparison of the T_m values for the mutually complementary dodecamers in the complexes meta-dA_{12}/meta-dU_{12} and genomic dA_{12}/dT_{12} indicates that the genomic complex is more stable.[16] However, the opposite behavior is observed for the self-complementary dodecamers meta-d$(AU)_6$ and genomic d$(AT)_6$. Here, a comparison of the T_m values indicates that the meta dodecamer structure is the more stable.

If one accepts the premise that an RNA or RNA-like polymer served a central role in the origins of life, then there is a need to reconcile the biotic predominance of the 3′,5′-internucleotide linkage with the likely prebiotic bias toward a 2′,5′-linkage. One way to do this would be to postulate an ancestral nucleic acid that contained a sugar different from ribose.[17] An alternative scenario would consider 2′,5′-linked nucleotides to be ancestral to genetic material composed of 3′,5′-linked nucleotides. On the basis of the findings reported in this work, there is no apparent reason why 2′,5′-linked nucleotides could not both replicate accurately and assume a tertiary structure conducive to catalysis.[4] Evidence for these possibilities awaits further experiments.

In summary, the self-associative property of 3′,5′-linked nucleotides is shared by 2′,5′-linked ones. As a consequence, any scenario in which the latter precede the former during prebiotic or biotic evolution would appear reasonable, but will require an explanation for natural selection[18] of the present-day genomic linkage.

Acknowledgment. Acknowledgement is made to the National Institutes of Health (GM-47375), the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the University of California for support of this research.

(13) Lerner, D. B.; Kearns, D. R. *Biopolymers* **1981**, *20*, 803.
(14) Young, P. R.; Kallenbach, N. R. *J. Mol. Biol.* **1978**, *126*, 467.
(15) A similar control experiment is not possible for the self-complementary meta-DNA dodecamer. When the dodecamer concentration is varied by 1 order of magnitude, the T_m does not change, consistent with a monomolecular hairpin structure.
(16) The stability of the meta complex relative to the genomic one is actually greater than simple comparison would suggest, as the stability of the genomic complex is enhanced by the hydrophobic methyl groups in the dT residues, which the dU residues of the meta complex lack. See: Zmudzka, B.; Bollum, F. J.; Shugar, D. *J. Mol. Biol.* **1969**, *46*, 169.
(17) Joyce, G. F.; Schwartz, A. W.; Miller, S. L.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4398.
(18) One probable selective advantage of the RNA 3′,5′-internucleotide linkage would be a greater stability than the 2′,5′-linkage toward hydrolysis when in a right-handed helical conformation, see: Usher, D. A. *Nature (London), New Biol.* **1972**, *235*, 207. Usher, D. A.; McHale, A. H. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 1149; this work does not address the stability of a 2′,5′-linkage in a purely, or a predominantly, 2′,5′-linked RNA strand, however.

Stereochemistry of Enzyme-Catalyzed Decarboxylation of α-Methyl-α-phenylmalonic Acid

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We have recently demonstrated a highly enantioselective and effective decarboxylation of α-methyl-α-phenylmalonic acid (**1**) by *Alcaligenes bronchisepticus* KU 1201 into (*R*)-α-phenylpropionic acid (**2**).[1] Because this was the first example of enantioselective enzymatic decarboxylation of a synthetic substrate,[2] the stereochemical course of the reaction is of great interest. Two substrate enantiomers **1** containing ^{13}C at either one of the two carboxyl groups were synthesized. The starting material, [1-^{13}C]phenylacetic acid (**3**) (99% ^{13}C), was commercially available.

As shown in Scheme I, methylation of **4** followed by benzyloxymethylation and deprotection afforded [1-^{13}C]-α-methyltropic

(1) (a) Miyamoto, K.; Ohta, H. *J. Am. Chem. Soc.* **1990**, *112*, 4077. (b) Miyamoto, K.; Ohta, H. *Biocatalysis* **1991**, *5*, 49.
(2) Although some efforts have been made by chemical methods, enantiomeric excesses of the products were very low: (a) Toussaint, O.; Capdevielle, P.; Maumy, M. *Tetrahedron Lett.* **1987**, *28*, 539. (b) Verbit, L.; Halbert, T. R.; Patterdon, R. B. *J. Org. Chem.* **1975**, *40*, 1649.

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