

A Novel Copper-Mediated DNA Base Pair

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DNA encodes information in the sequence of the four deoxy-nucleotides dA, dT, dG, and dC. The key element for information storage and retrieval in this four-letter alphabet involves selective hydrogen bonding in the complementary base pairs dA:dT and dG:dC. Are these universal bases a unique structural solution for the genetic code, or are there other base pairing schemes that can be incorporated into DNA? To address this question, we and others have begun to synthesize and evaluate base pairs with alternate pairing interactions. The ability to generate additional such pairs would not only increase the capacity of DNA for information storage¹ but would also allow the replication of DNA containing novel functional groups.² In addition, nucleic acids completely built out of artificial base pairs could lead to novel polymers with interesting chemical and physical properties.

Efforts to expand the genetic alphabet have focused largely on two strategies: hydrogen bonding patterns different from those in dA:dT and dG:dC³ and hydrophobic packing interactions.^{4,5} Here we describe a new strategy which is based on the metal-dependent pairing of two nucleobases.^{6,7} We have designed a base pair with a pyridine-2,6-dicarboxylate nucleobase (Dipic) as a planar tridentate ligand, and a pyridine nucleobase (Py) as the complementary single donor ligand (Figure 1). This [3 + 1] square planar coordination was chosen over a [2 + 2] coordination geometry since the chelating effect of the tridentate ligand should give rise to a higher metal ion affinity in comparison to that of the nitrogen atoms of the natural nucleobases.⁸ The [3 + 1]

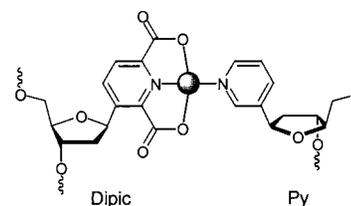


Figure 1. Base pairing between Dipic and Py by complexation to a metal ion.

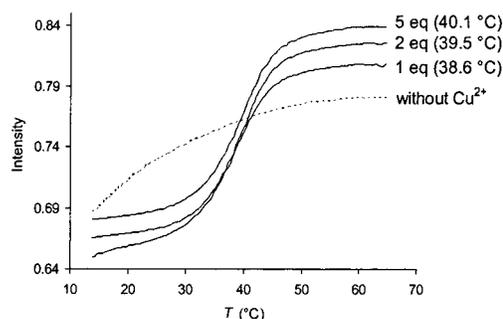


Figure 2. Melting curves of the duplex d(5'-CACATTADipicTGT-TGTA-3') and d(3'-GTGTAATPyACAACAT-5'), with and without different concentrations of Cu(NO₃)₂. Measurements were performed in 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7.0 with 2 μM DNA duplex.

coordination scheme also ensures that the metal ion is bound more tightly to one of the two unnatural nucleobases, which may be important for enzymatic replication. In addition, metal–ligand coordinative bonds have bond energies of 10–30 kcal/mol, intermediate between covalent bonds and noncovalent interactions such as hydrogen bonds. Consequently, one metal–ligand bond should be able to compensate for two or three hydrogen bonds as found in the natural base pairs dA:dT and dG:dC, respectively.

Nucleosides bearing a pyridine base and a 2,6-pyridinedicarboxylic methyl diester⁹ were synthesized by the method of Eaton and Millican¹⁰ and converted into the respective phosphoramidites. Incorporation into oligodeoxynucleotides and deprotection of the fully protected products were carried out with standard protocols using an automated DNA synthesizer. To examine the influence of metal ions on the thermal stability of the Dipic:Py base pair in DNA, the pair was introduced in the middle of a 15-nucleotide duplex, and the melting temperature (T_M) was determined by UV-monitored thermal denaturation. To remove trace transition metal ions, oligonucleotide solutions were treated with a chelating resin (Chelex 100 from Sigma). In the absence of transition metal ions, a 1:1 mixture of oligonucleotides (2.0 μM each) containing Dipic and Py showed no melting transition between 14 and 65 °C, indicating that the two complementary oligonucleotides are dissociated, even at 14 °C (dotted line in Figure 2).

For comparison, the duplex containing a dA:dT versus Dipic:Py base pair melts at 41.2 °C. Thus, in the absence of transition metal ions the Dipic:Py base pair strongly destabilizes the duplex structure. Addition of metal salts CeCl₃, Mn(NO₃)₂, Fe(SO₄)₂, Co(NO₃)₂, Ni(NO₃)₂, Zn(NO₃)₂, Pd(NO₃)₂, and K₂PtCl₄ afforded no increase in duplex stabilization, even with a large excess.

In contrast, addition of 1 equiv of Cu²⁺ ions led to base pairing with comparable thermal stability to the natural dA:dT base pair—the Dipic:Py base pair resulted in a biphasic melting curve with a melting point of 38.6 °C. Additional Cu²⁺ ions further increase

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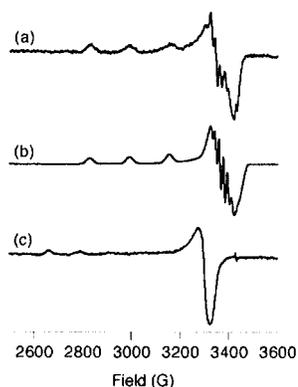


Figure 3. (a) EPR spectrum (9.615 MHz) of a frozen (20 K) solution of d(5'-CACATTG**Dipic**TGTTGTA-3') (100 μ M), d(3'-GTGTAAC**Py**A-CAACAT-5') (100 μ M), Cu(NO₃)₂ (100 μ M), sodium phosphate (5 mM), and NaClO₄ (50 mM) at pH 7.0, with 10% glycerin. (b) Simulation (QPOWA) using the spin Hamiltonian parameters described in the text. (c) EPR spectrum of a frozen (20 K) solution of CuSO₄ (110 μ M), HCl (0.095 M), and NaClO₄ (1.03 M), with 10% glycerin.

the stability only slightly (Figure 2). This strong metal-dependent stabilization is not found with the other square planar geometry favoring metal ions Ni²⁺, Pd²⁺, and Pt²⁺. Cu²⁺ has the highest complex formation constants relative to the other first row transition metal divalent ions—the formation constant for the 1:1 complex of Cu²⁺ with pyridine-2,6-dicarboxylate is 1.4×10^9 mol⁻¹, 2 orders of magnitudes higher than the corresponding constants for Zn²⁺ (1.9×10^7 mol⁻¹) and Ni²⁺ (2.8×10^7 mol⁻¹).¹¹ The greater complex formation constant for Cu²⁺ complexes also leads to stronger coordination to the pyridine base in the complementary strand (formation constants for the 1:1 M²⁺/pyridine complexes: 363 mol⁻¹ for Cu²⁺, 73 mol⁻¹ for Ni²⁺, 11.5 mol⁻¹ for Zn²⁺),¹² which is presumably the main factor for stabilizing the duplex structure. Pd²⁺ and Pt²⁺, on the other hand, have larger ionic radii and likely do not fit as well into the binding site of the tridentate ligand.¹³ Additionally, these softer transition metal ions have a much lower tendency to coordinate carboxylate ligands and thus probably prefer binding to the nitrogen atoms of the natural bases.

Electron paramagnetic resonance (EPR) was used to verify the presence of bound Cu²⁺ and as a qualitative structural probe. Figure 3(a) shows the X-band EPR spectrum of a solution of the Dipic:Py-containing duplex with 1 mol equiv of Cu²⁺.

The signal integrates to 98% of the expected value, and is indicative of a single Cu²⁺ compound. Comparison to (c), the spectrum of a control solution of Cu²⁺, reveals that the copper ions are quantitatively bound in a substantially different environment in the presence of the oligodeoxynucleotides. The spin Hamiltonian parameters for the unpaired electron, derived from simulation of the spectrum as shown in Figure 3(b), are $g_x = 2.034$, $g_y = 2.047$, $g_z = 2.236$; $A_{x,y}(^{14}\text{N}) = 17 \times 10^{-4}$ cm⁻¹, $A_z(^{63,65}\text{Cu}) = 170 \times 10^{-4}$ cm⁻¹. This slightly rhombic signal, with $g_z > g_{x,y} > g_e$, is consistent with an electron in a $d_{x^2-y^2}$ orbital in a copper complex of square-planar or square-pyramidal geometry. The successful simulation of the superhyperfine coupling to two ¹⁴N nuclei supports the proposed coordination of two nitrogen and two oxygen ligands. In addition, the g_z and A_z values are within the range expected for N₂O₂ ligation.¹⁴ Our values are

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Table 1. Melting Temperatures of Mismatches with Dipic and Py^a

5'-C-A-C-A-T-T-A-X-T-G-T-T-G-T-A-3' 3'-G-T-G-T-A-A-T-Y-A-C-A-A-C-A-T-5'					
X:Y	T _M [°C] ^b	X:Y	T _M [°C] ^b	X:Y	T _M [°C] ^b
Py:Dipic	40.4	Dipic:C	30.5	C:Py	<i>c</i>
Dipic:Dipic	<i>c</i>	Py:Py	28.0	A:T	41.1
DipicA:	36.1	Py:A	28.5	A:G	31.5
DipicT:	<i>c</i>	Py:T	29.2	C:C	23.5
DipicG:	28.6	Py:G	26.9		

^a Measured in 5 mM sodium phosphate, 50 mM sodium perchlorate, pH 7.0 with 30 μ M Cu(NO₃)₂ and 2 μ M DNA duplex. ^b Melting temperatures are mean values from heating and cooling curves of two independent experiments. Errors are estimated to be within ± 0.2 °C. ^c No sigmoidal melting curve was observed in the temperature range between 15 and 65 °C.

somewhat different from those reported for the simple coordination compound (Dipic)Cu(py) ($g_x = 2.052$, $g_y = 2.080$, $g_z = 2.280$; $A_{x,y}(^{14}\text{N}) = 15 \times 10^{-4}$ cm⁻¹, $A_z(^{63,65}\text{Cu}) = 184 \times 10^{-4}$ cm⁻¹).¹⁵ These relatively small deviations may be due to a difference in geometry, magnetic anisotropy from the surrounding nucleobases, and/or a solvent effect. Thus, the EPR spectral data are consistent with the proposed binding mode of the Cu²⁺ to the Dipic:Py base pair.

To test the Cu²⁺-dependent pairing specificity of Dipic and Py, the melting temperatures of duplexes containing mismatches with the natural bases were measured. The data in Table 1 show that the duplex containing the Dipic:Py base pair is considerably more stable than those containing mismatches with one of the natural bases. Relative to Dipic:Py, the mispairs are destabilized by 10 °C or more, except the mispair between Dipic and dA which is destabilized by 4.3 °C. For comparison, mispairs between the natural nucleobases are all destabilized by at least 10 °C in this sequence context. Thus, although the discrimination of the natural bases against mispairs is higher, these results clearly demonstrate that metal-assisted base pairing can be quite selective.

The above results demonstrate that interbase metal coordination can replace the hydrogen bonding schemes found in the natural base pairs dA:dT and dG:dC. All plants, animals, and bacteria use copper; after iron and zinc, copper is the third most abundant transition metal in the human body with concentration in the micromolar range.¹⁶ This makes it a very attractive cofactor for the expansion of the genetic code in vivo. Finally, the construction of oligomers consisting entirely of Dipic:Py could lead to materials with interesting electronic properties based on the controlled and periodic spacing of copper ions along the helix axis.

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Supporting Information Available: Synthesis scheme for the nucleosides, Dipic and Py, and experimental procedure for the synthesis of the modified deoxyoligonucleotides (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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