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Pd^{2+}-mediated base pairing in oligonucleotides

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ABSTRACT

Two short glycol nucleic acid (GNA) oligonucleotides, having either a terminal or an intrachain nucleobase replaced by the pyridine-2,6-dicarboxamide chelate of Pd^{2+}, have been synthesized and their hybridization properties studied by melting temperature measurements. In the termini of a double-stranded oligonucleotide, the Pd^{2+} chelates provided dramatic stabilization of the duplex relative to its metal-free counterpart, in all likelihood owing to formation of Pd^{2+}-mediated base pairs between pyridine-2,6-dicarboxamide and the opposing nucleobase. In contrast, no stabilization was observed when the Pd^{2+} chelate was placed in the middle of the chain. Furthermore, the results could not be reproduced by adding a Pd^{2+} salt *in situ* to the dilute oligonucleotide solutions but the palladated oligonucleotides had to be synthesized and purified prior to the hybridization studies. This behavior, presumably attributable to the relatively slow ligand-exchange reactions of Pd^{2+}, differs greatly from what is usually observed with more labile metal ions. The present results offer an explanation for the failure of previous attempts to incorporate Pd^{2+}-mediated base pairs into oligonucleotides.

1. Introduction

Metal-ion-mediated base pairing of nucleic acids has been studied extensively for more than a decade owing to its potential in DNA nanotechnology and in expanding the genetic alphabet [1–9]. So far, the most promising results have been obtained with the kinetically labile Ag^{+}, Hg^{2+} or Cu^{2+} as the bridging metal ion. For expanding the range of applications of metal-ion-mediated base pairing to chemotherapy, however, the low intracellular concentrations of these metal ions present a severe limitation. DNA cross-linking by the kinetically inert Pt^{2+}, in turn, is a widely employed strategy in cancer chemotherapy [10–12] but suffers from low sequence-specificity. Oligonucleotides capable of metal-ion-mediated hybridization even under the metal-deficient conditions of the cell would offer an elegant solution to this problem.

Along with Pt^{2+}, Pd^{2+} appears an ideal candidate for metal-ion-mediated base pairing. Both of these metal ions prefer nitrogen over oxygen ligands and form highly stable square-planar complexes that are compatible with the steric requirements of the base stack of a double-helical nucleic acid. At monomeric level, several Pd^{2+}-mediated base pairs have been described in the literature [13–19]. In fact, the very first artificial metal-ion-mediated base pair was formed through coordination of Pd^{2+} [20]. Incorporation of a Pd^{2+}-mediated base pair into an oligonucleotide, however, remains to be convincingly demonstrated. The apparent discrepancy between the results obtained at monomer and oligomer levels may be understood in terms of the relatively slow ligand exchange reactions of Pd^{2+}. A double-helical oligonucleotide presents many potential donor atoms and a Pd^{2+} ion introduced *in situ* may well remain kinetically trapped at a nonproductive binding site.

In this article we present compelling evidence for Pd^{2+}-mediated base pairing within an oligonucleotide. K_2PdCl_4 was allowed to react with short glycol nucleic acid (GNA) oligonucleotides incorporating a single high-affinity ligand. The GNA backbone was chosen as a test bed because of the relatively easy and facile synthesis of modified GNA building blocks. The metallated oligonucleotides were purified by RP HPLC before hybridization experiments. Coordination of a single Pd^{2+} ion by the modified oligonucleotides was verified by mass spectrometric analysis. Thermal melting studies with these oligonucleotides revealed the Pd^{2+}-mediated base pair to be better tolerated in a terminal than in an internal position.

2. Results and discussion

2.1. Building block synthesis

The GNA phosphoramidite building blocks of the canonical nucleobases were prepared as described previously [21–23]. For preparation of a respective building block of the metal-ion-chelating residue (**1**), the phenolic OH function of diethyl 4-hydroxypyridine-2,6-dicarboxylate was first derivatized by treatment with (*R*)-2,2-dimethyl-1,3-dioxolane-4-methanol *p*-toluenesulfonate (Scheme 1). The isopropylidine protection was then removed under acidic conditions and the liberated primary OH function was protected as a 4,4′-dimethoxytrityl ether. The secondary OH of the protected intermediate

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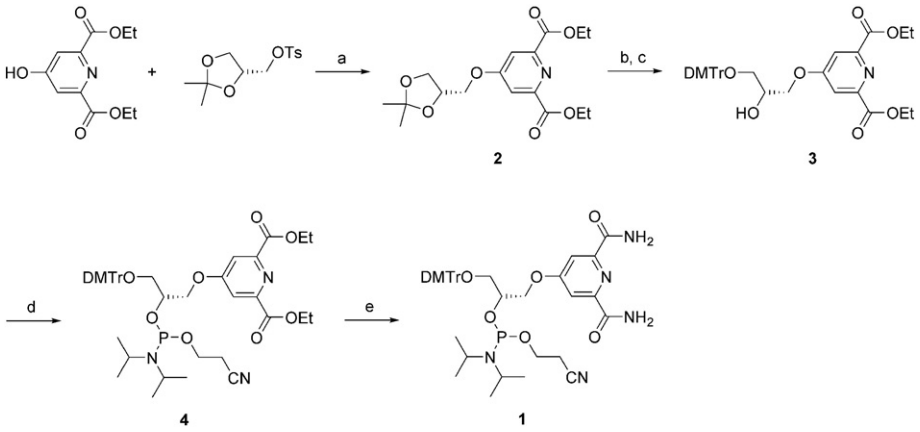


Scheme 1. Synthesis of the modified GNA phosphoramidite building block **1**. Reagents and conditions: (a) K_2CO_3, DMF; (b) HCl, MeCN, H_2O; (c) DMTrCl, pyridine; (d) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite, Et_3N, CH_2Cl_2; (e) NH_3, MeOH.

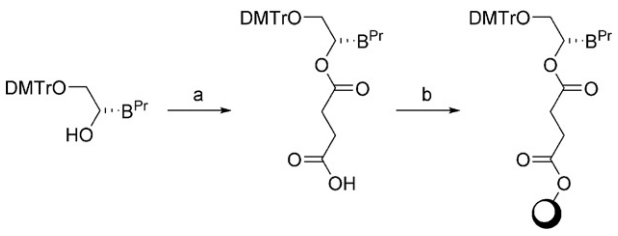
3 was phosphitylated by conventional methods. Finally, the ethoxycarbonyl substituents of the pyridine ring were converted to aminocarbonyl substituents by treatment with methanolic ammonia to afford the phosphoramidite building block **1**. It is worth pointing out that GNA oligonucleotides with free OH termini will undergo cleavage under these conditions.

2.2. Preparation of solid supports for the synthesis of GNA oligonucleotides

For immobilization on solid support, protected GNA counterparts of the canonical 2′-deoxynucleosides were first succinylated by treatment with succinic anhydride in anhydrous pyridine (Scheme 2). The crude products were then attached to long chain alkylamine-functionalized controlled pore glass (LCAA-CPG) by conventional 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU)-promoted peptide coupling. Based on trityl response on treatment with 3% dichloroacetic acid in CH_2Cl_2, loadings of the solid supports thus obtained were found to be in the typical range (30–50 μmol g^{-1}).

2.3. Oligonucleotide synthesis

The oligonucleotides (Table 1) were assembled by an automated synthesizer on a CPG-supported succinyl linker. Standard phosphoramidite strategy with 300 s coupling time (with 5-benzylthio-1*H*-tetrazole as the activator) was used throughout the sequences. Based on the trityl response, the coupling yields were 44% for the modified building block **1** and more than 98% for the other building blocks. The oligonucleotides were released from the supports and the phosphate and base protections removed by treatment with either 16% aq. ammonia (for **ON1** and **ON2**) or 20% aq. methylamine (for **ON3**) for 35 min at 55 °C. To minimize cleavage of the phosphodiester linkages, the aminolyses were carried out with the 3′-*O*-(4,4′-dimethoxytrityl) (DMTr) protection in place. This protecting group was finally removed in 80% aq. acetic acid (60 min at room temperature).



Scheme 2. Preparation of solid supports for the synthesis of GNA oligonucleotides. Reagents and conditions: (a) succinic anhydride, DMAP, pyridine; (b) LCAA-CPG, HBTU, DIPEA, DMF.

The crude oligonucleotides were purified by RP-HPLC and the purified products characterized by electrospray ionization mass spectrometry (ESI-MS). The concentrations of the oligonucleotides were estimated UV-spectrophotometrically based on absorbance at 260 nm. The molar absorptivities were calculated by an implementation of the nearest-neighbors method assuming the hypochromicities of GNA and DNA oligonucleotides to be equal [24,25]. For determination of the molar absorptivity of the modified residue, compound **2** was deprotected and converted into the diamide **5** by successive treatments with hydrochloric acid and methanolic ammonia (Scheme 3). Molar absorptivity of **5** at 260 nm was determined to be 2000 M^{-1} cm^{-1} (UV spectrum presented as supporting information).

2.4. Preparation of Pd^{2+}-carrying oligonucleotides

Oligonucleotide **ON1**, bearing the modified residue **5** in its 5′-terminus, was dissolved in 2 mM aq. K_2PdCl_4, to a final concentration of 1.3 mM. This mixture was incubated overnight at room temperature, after which it was diluted with phosphate buffer (pH 7.2). The product mixture was fractioned by RP-HPLC eluting with an MeCN gradient in phosphate buffer and the collected fractions desalted by RP-HPLC eluting with an MeCN gradient in water. Based on the HPLC profile, **ON1** had been converted nearly quantitatively to products of lower mobility (Fig. 1). ESI-MS analysis revealed the main product, eluting at 12.5 min, to be a complex of two **ON1** molecules and two Pd^{2+} ions (Fig. 2, $\mathbf{ON1}_2$:Pd_2). Each of the Pd^{2+} ions had displaced two protons, consistent with the binding mode reported previously for pyridine-2,6-dicarboxamide [18]. Given the self-complementarity of the tetranucleotide sequence in the middle of **ON1**, a homoduplex with terminal Pd^{2+}-mediated base pairs between the pyridine-2,6-dicarboxamide moiety of the modified residue **5** and thymine appears the most likely structure for $\mathbf{ON1}_2$:Pd_2. Coordination of Pd^{2+} to the N3

Table 1
Structures of the GNA oligonucleotides used in this study.

	Sequence
ON1	3′-**X**CGCGT-2′
ON2	3′-CGC**X**GGC-2′
ON3	3′-GCCTGCG-2′

X =