Synthesis and Hybridization Properties of Covalently Mercurated and Palladated Oligonucleotides

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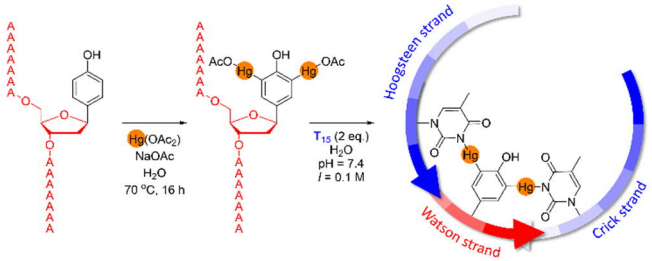

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Abstract Covalent metalation of the base moieties affords a new class of modified oligonucleotides. These organometallic oligonucleotides share many properties, notably increased hybridization affinity conferred by metal-mediated base pairing, with oligonucleotides incorporating coordinative transition metal complexes. They are, however, set apart by their ability to retain the transition metal ion even at extreme dilution. Such stability towards dissociation would be desirable in DNA nanotechnology and necessary in therapeutic applications. Herein we describe our efforts towards preparation and characterization of covalently mercurated and palladated oligonucleotides, highlighting in particular our recent contribution on the synthesis and potential applications of oligonucleotides incorporating dimercurated artificial nucleobases.

Key words organometallic; oligonucleotide; mercury; palladium; hybridization

1 Introduction

Interaction of nucleic acids with transition metal ions mainly takes place through coordination to the endocyclic nitrogen atoms of the nucleobases (N1 and N7 of purines and N3 of pyrimidines).[1] Cisplatin and related platinating agents are probably the best-known application of this coordination, dating back to the 1960s[2] and still playing a key role in cancer chemotherapy.[3] While intrachain cross-linking of two bases by cisplatin disrupts the secondary structure of the nucleic acid, interchain cross-linking by many other metals has been shown to be quite stabilizing and compatible with the geometry of the double helix. The earliest example of this metal-mediated base pairing was reported already in 1963[4] although the term was not coined until the early 2000s.[5] Since then, an increasing number of applications of metal-mediated base pairing between natural as well as artificial nucleobases have been proposed, including sensors, molecular wires, nanoparticles and sequence recognition.[6]

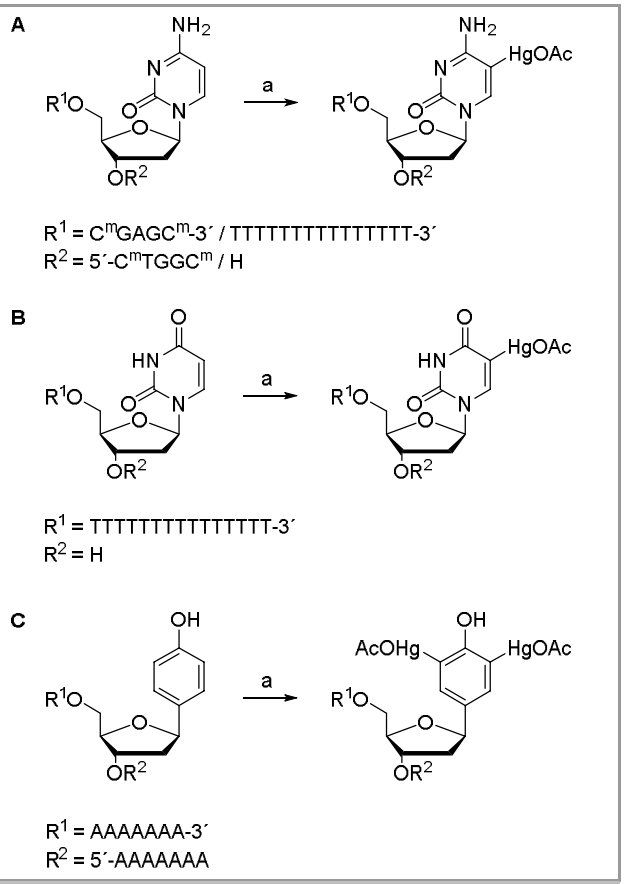
Although not studied as extensively as coordination to the electronegative donors, binding of transition metals to the carbon atoms of nucleobases was first reported as early as 1973.[7] Direct covalent metalation of natural nucleobases is, to the best of our knowledge, limited to mercury[8] but the scope has been widened by introduction of aryl substituents to position 6 of a purine base.[9] Covalently metalated oligonucleotides have the benefit of resisting dissociation even in highly metal-deficient environments, such as the intracellular medium.

The requirement for stability of the M-C bond under physiological conditions obviously limits the number of metals usable in covalently metalated oligonucleotides designed for biological applications but at least mercury, gold and the platinum group metals appear viable candidates. We have recently become interested in the synthesis of covalently mercurated and palladated oligonucleotides, with the ultimate goal of harnessing the superior affinity of metal-mediated base pairing for sequence recognition in biological media.

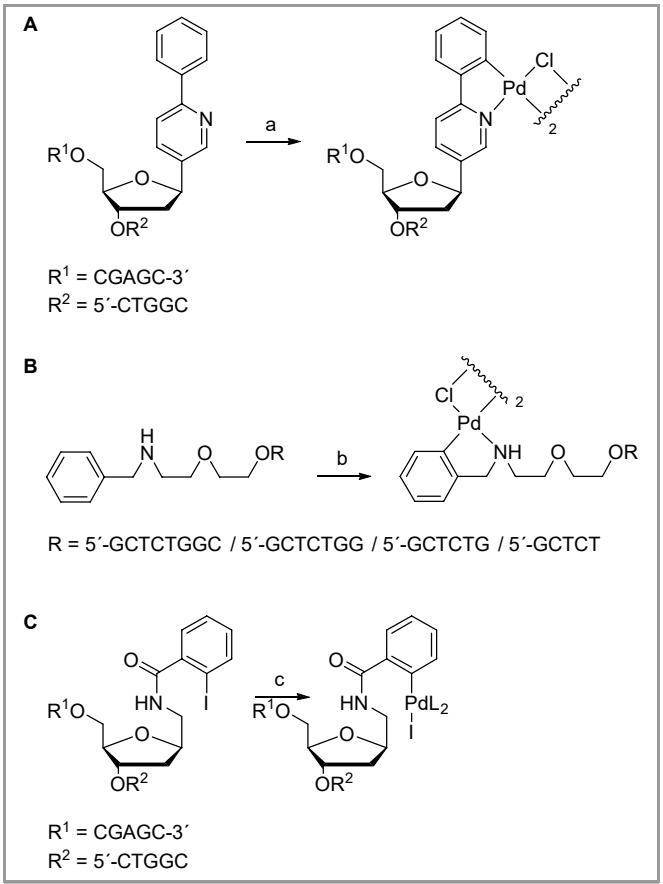
2 Synthesis of covalently mercurated and palladated oligonucleotides

The relatively electron-rich C5 carbons of cytosine and uracil bases are readily mercurated by Hg(II) salts under mild conditions (Scheme 1A and B).[8] In an oligondeoxynucleotide, mercuration can be limited to the desired cytosine residues by using 5-methylcytosine in place of all the others.[11] Besides cytosine and uracil, artificial bases such as phenol[12] can also be mercurated by the same method provided that they are sufficiently electron-rich. In the case of phenol, the hydroxy substituent directs the reaction to the ortho carbons (the para carbon being engaged in the C-glycosidic bond) and it is actually possible to mercurate both of these carbons (Scheme 1C) although the second mercuration is sluggish. With long-running treatments, precipitation of Hg(II) or – depending on the sequence – the oligonucleotide may present a problem.

In contrast to mercuration, palladation of a C-H bond under conditions where oligonucleotides remain otherwise intact requires a directing ligand – a proximal atom with a lone pair for coordinating Pd(II). Representative structures include phenylpyridines and benzylamines (Scheme 2A and B).[13] Cyclopalladation of oligonucleotides featuring such "hot spots" is accomplished under mild conditions with a small excess of a Pd(II) salt.[14, 15] As none of the canonical nucleobases are amenable to cyclopalladation the synthesis of cyclopalladated oligonucleotides invariably starts with the synthesis of phosphoramidite building blocks of appropriate artificial nucleoside analogues.



Scheme 1 Direct mercuration of natural and artificial nucleobases. Reagents and conditions: a) $HgOAc_2$, NaOAc, H_2O.



Scheme 2 Ligand-directed cyclopalladation of A) phenylpyridine and B) benzylamine residues and C) oxidative addition of Pd(0) to an iodoaryl residue. Reagents and conditions: a) Li_2PdCl_4, NaOAc, MeOH, H_2O; b) Li_2PdCl_4, MeCN, H_2O; c) $Pd_2(dba)_3$, MeCN, H_2O, Ar atmosphere.

Oxidative addition of Pd(0) to an iodoaryl residue offers another method for covalent palladation of oligonucleotides (Scheme 2C). The obvious advantage of this strategy is that, without the need for a directing ligand, the structure of the palladated nucleobase surrogate can be designed more freely and steric crowding around the palladium center can be reduced. We have only recently started to explore the potential of this approach but initial results have been promising.[16]

Chromatographic purification of covalently mercurated oligonucleotides is complicated by their tendency to form aggregates. This problem manifests itself in broad peaks comprising both unmercurated and mercurated oligonucleotides and is especially pronounced with oligonucleotides featuring multiple sites of mercuration. Two or three passes through an RP-HPLC column are typically needed to obtain a pure product.

The chromatographic profiles of palladated oligonucleotides differ from those of mercurated oligonucleotides in that several discrete peaks are typically observed, rather than a single broad one. This difference likely stems from the slower ligand-exchange of Pd(II) compared to Hg(II). Even when collected separately, the peaks are indistinguishable by mass spectrometric analysis (the exchangeable ligands are usually not seen in the mass spectrum) and, given enough time, will equilibrate. In general, palladated oligonucleotides tend to be easier to purify than their mercurated counterparts owing to higher metalation yields and smaller excess of the metal salt in the reaction mixture.

3 Hybridization properties of covalently mercurated and palladated oligonucleotides

Hybridization of covalently mercurated oligonucleotides with unmodified complementary sequences can be studied by conventional UV melting experiments. Ligand-exchange of Hg(II) is so rapid that smooth sigmoidal denaturation and renaturation curves with negligible hysteresis are typically obtained even when Hg(II)-mediated base pairing is involved. Thermal denaturation of duplexes incorporating Hg(II)-mediated base pairs is, however, significantly more gradual compared to duplexes comprising only hydrogen-bonded base pairs. Gradual melting indicates a relatively small negative entropy of hybridization, attributed to the loss of the solvation shell of Hg(II) on being embedded within the base stack of a double helix.[17] In other words, stabilization of an oligonucleotide duplex by a Hg(II)-mediated base pair is largely due to a decreased entropic penalty of hybridization, rather than increased bonding enthalpy.

Consistent with the tendency of Hg(II) to displace relatively acidic protons, the strongest Hg(II)-mediated base pairs are usually formed with guanine, thymine and uracil (pK_a = 9.5, 10.0 and 8.9 for GMP-N1, TMP-N3 and UMP-N3, respectively). This general trend can be observed with individual Hg(II)-mediated base pairs by NMR as well as with the corresponding mercurated duplexes by UV melting experiments (Figure 1).[11, 12] Interestingly, with 2,6-dimercuriphenol-modified homoadenine oligonucleotides, duplexes placing an adenine or a cytosine

residue opposite to the mercurated residue also exhibited higher melting temperatures than a canonical homoadenine•homothymine duplex of the same length. Nevertheless, the duplex pairing the mercurated residue with thymine was still more stable by a nearly 10 °C margin.

Hg(II) can also coordinate to the N7 on the "Hoogsteen face" of purine nucleobases[1] so enhancing the hybridization properties of triplex-forming oligonucleotides by Hg(II)-mediated Hoogsteen-type base pairing appears a plausible strategy. Our first experiments with triplex-forming oligonucleotides bearing a 3′-terminal 5-mercuricytosine residue indeed revealed increased Hoogsteen as well as Watson-Crick melting temperatures with A•T or T•A as the target base pair.[18] The origin of this stabilization remained obscure, however, as the effect was actually amplified in the presence of 2-mercaptoethanol, a strong competing ligand for Hg(II).[19]

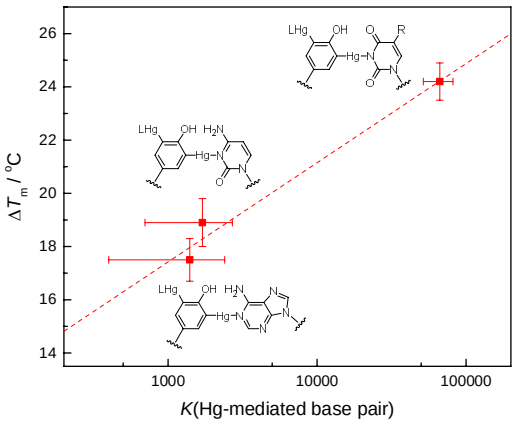


Figure 1 Stabilization of oligonucleotide duplex (ΔT_m) on introduction of a Hg(II)-mediated base pair correlates with stability of the base pair in question (determined independently by NMR).[12]

The two Hg(II) ions of 2,6-dimercuriphenol lie on the "Watson-Crick" and "Hoogsteen" faces of this artificial nucleobase and allow formation of another kind of triple helix, namely one where the central homopurine strand bears the mercurated residue.[12] This binding mode proved highly promising, with both Hoogsteen and Watson-Crick melting temperatures of triplexes incorporating a single 2,6-dimercuriphenol being more than 10 °C higher than the respective values of unmodified triplexes of the same length (Figure 2). Examples of naturally occurring pathogenic nucleic acids that could be targeted in this way include the viral transcript polyadenylated nuclear (PAN) RNA and related structures.[20] Furthermore, the fact that stabilization was observed not only with thymine but also with adenine as the base-pairing partner of 2,6-dimercuriphenol suggests the possibility of invasion of AT-rich double helices with mercurated oligonucleotides.

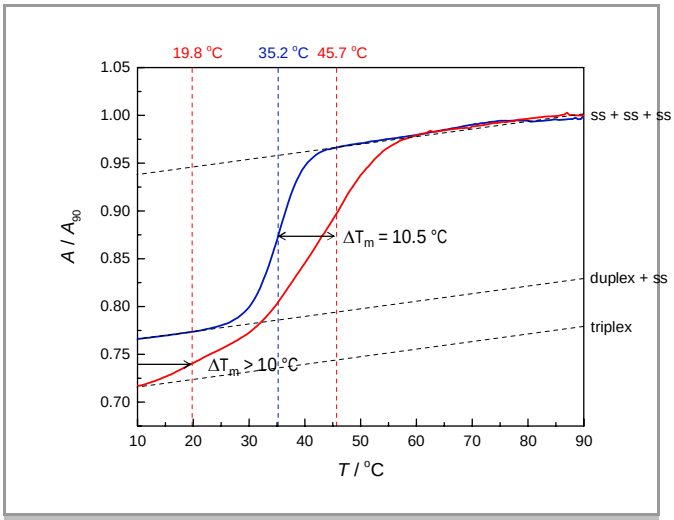


Figure 2 UV melting profiles for 15-mer homothymine*homoadenine•homothymine triplexes featuring either adenine (blue) or 2,6-dimercuriphenol (red) in the middle of the homoadenine strand (in the former case, triplex formation is hardly detected).[12]

The relatively slow ligand-exchange of Pd(II) makes hybridization studies with covalently palladated oligonucleotides by conventional methods much more challenging than with their covalently mercurated counterparts. The UV denaturation curves typically exhibit several overlapping transitions and considerable hysteresis between the denaturation and renaturation curves is also commonly observed.[14, 16] Furthermore, while the square planar coordination sphere of Pd(II) is in principle amenable to Pd(II)-mediated base pairing within a double helix, finding an optimal structre is more difficult than with metals favoring linear cooridnation. It is even possible for a Pd(II)-mediated base pair itself to persist at the high end of the temperature ramp of a UV melting experiment (90 °C) and yet disrupt the pairing of neighboring canonical bases to such an extent that the apparent melting temperature of the duplex is actually lower than in the absence of Pd(II)-mediated base pairing. As expected, such disruption is more pronounced in the middle of the base stack[14] than at a terminal position.[15]

4 Outlook

Covalent metalation is an as yet underexploited approach for introducing functionality to oligonucleotides. Our interest has mainly focused on metal-mediated base pairing of covalently mercurated and palladated oligonucleotides but organometallic oligonucleotides could also find use as asymmetric catalysts or, possibly, artificial nucleases. Perhaps the most remarkable advantage of organometallic complexes over their coordinative counterparts is their resistance to dissociation even in metal-deficient media and at extreme dilution. Such stability would be particularly important in biological applications given the scarcity of available transition metal inside the cell.

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Biosketches

Tuomas Lönnberg (second from the right) was born in Turku, Finland. He received his M.Sc. from the University of Turku in 2001 and his Ph.D. from the same university in 2005 under the supervision of Professor Satu Mikkola. From 2006 to 2008, he worked as a JSPS Post-Doctoral Fellow in the group of Professor Makoto Komiyama at the University of Tokyo. Since 2008, he has held various research and teaching positions at the University of Turku. In 2013 – 2014, he visited the laboratory of Professor Steven Rokita at Johns Hopkins University as an ASLA-Fulbright Senior Scholar. In 2016, Tuomas Lönnberg was appointed Assistant Professor of organic chemistry at the University of Turku. His research interests include covalently metalated oligonucleotides and biologically relevant phosphate-transfer reactions.

Sajal Maity (second from the left) was born in Howrah, India. He received his B.Sc. and M.Sc. from Calcutta University in 2001 and 2004, respectively and his Ph.D. from Jadavpur University in 2012 and is currently working as a post-doctoral fellow in the group of Dr. Tuomas Lönnberg.

Dattatraya Ukale (left) was born in Alsunde, India. He received his B.Sc. and M.Sc. from the University of Pune in 2008 and 2010, respectively, and is currently pursuing a Ph.D. under the supervision of Dr. Tuomas Lönnberg.

Madhuri Hande (right) was born in Pune, India. She received her B.Sc. and M.Sc. from Savitribai Phule Pune University in 2012 and 2014, respectively, and is currently pursuing a Ph.D. under the supervision of Dr. Tuomas Lönnberg.