Ligand-based backbone modifications for metal-chelating nucleic acids†

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Ligands were incorporated into the backbone of DNA for nucleoside replacements, and the binding of metal ions, such as Cu^{2+}, Pt^{2+} and Pd^{4+}, was shown to influence stability of the resulting duplexes.

The predictable and controlled structural properties of nucleic acids have inspired adaptation of these biomolecules beyond simple genetic information and toward antisense oligodeoxyribonucleotides (ODNs),[1] biosensors,[2,3] and nanoarchitectures.[4] Antisense nucleic acids are short segments of DNA or RNA designed to be complementary to a target RNA sequence. Binding of these ODNs to RNA can be used to suppress expression of an encoded protein. Easy synthetic access, paired with a nearly limitless adaptability for binding to specific RNA targets, has made anti-gene technology a promising tool for both analytical and pharmacological applications.[5,6] At this time, however, the majority of this promise has not yet been realized. With respect to drug design, significant limitations persist. In particular, the high charge of standard ODNs inhibits cellular uptake, and nuclease susceptibility minimizes the drug lifetime once inside cells. Consequently nucleic acid structures need to be altered in order to develop better drugs.

A wide variety of nucleic acid derivatives have been developed to address these limitations, including organically modified species such as peptide nucleic acids,[7] locked nucleic acids,[8] phosphorothioate DNA,[9] and morpholino-oligonucleotides.[10] Although these alterations have enhanced the efficacy of nucleic acids for both therapeutic and non-medical applications, greater utility remains hindered by toxicity and limited structural control. Introduction of metal–ligand chemistry can impart great flexibility to nucleic acids. The scope of inorganic chemistry has been invoked to expand the potential of DNA and RNA to design new materials, electron transfer assemblies, templates for organic reactions, artificial nucleases, labels for detection by electrochemical or fluorescence methods, and arrange metal ions in precise shapes for assembly of complex electronic devices.[11–15]

Incorporation of metal centers into nucleic acids has been achieved by many laboratories *via* four major strategies: unnatural metal-binding base pairs,[16–21] 5′- or 3′-pendant ligands,[15,22–26] sugar modifications,[27,28] and metal complexes tethered within the ODN backbone.[29–35] We are curious to see what influences on duplex stability can be brought about by metal complexes that play integral roles in the nucleic acid backbone structure. By using a variety of metals and ligands, a diverse collection of effects may result. In particular we are interested to see inorganic compounds incorporated into a single strand of DNA. Introducing a ligand to only one strand may afford the ability to bind naturally occuring DNA and RNA targets for applications such as antisense drug design and biosensor development.

Our efforts have focused upon replacing individual nucleosides with a variety of small metal-binding structures. In this approach, metal–ligand assemblies are included in a single strand of a DNA duplex for modulation of the ODN charge and structure. Standard base-pairing hydrogen bonding then directs annealing, allowing duplex formation with an unmodified complement. When complexed to a metal, these nucleoside replacements do not require a lengthy spacer or specialized nucleic acid structure for duplex formation. The incorporated ligands may bind positively charged metal ions thereby offsetting the negatively charged phosphate backbone. Metal binding to chelating oligodeoxyribonucleotides may thus influence and permit control of duplex stabilities. We have focused on three distinct ligands: bipyridine (Bpy), piperazine (Pip), and a dithioether (Dithio, Fig. 1). Bipyridine is a classic chelator. Piperazine[36–39] and dithioethers[40–43] are less common ligands but are known to chelate metal ions.

The Bpy ligand was prepared from 4,4′-dimethyl-2,2′-bipyridine which was deprotonated and then reacted with ethylene oxide (Fig. 2).[44] Subsequent addition of 4,4′-dimethoxytrityl chloride yielded a trityl-Bpy-alcohol.[45] The phosphoramidite was then obtained by treatment with 2–cyanoethyl-*N*,*N*,*N*′,*N*′-tetraisopropylphosphoramidite (Fig. 2).[46] The Pip and Dithio analogues were prepared in a similar manner (see ESI†). These ligand phosphoramidites were incorporated into oligodeoxyribonucleotides at various positions using standard automated synthetic techniques (see ESI†).[47] For each ligand, two different strands were synthesized: one in which nucleoside 12 of a 23-mer sequence

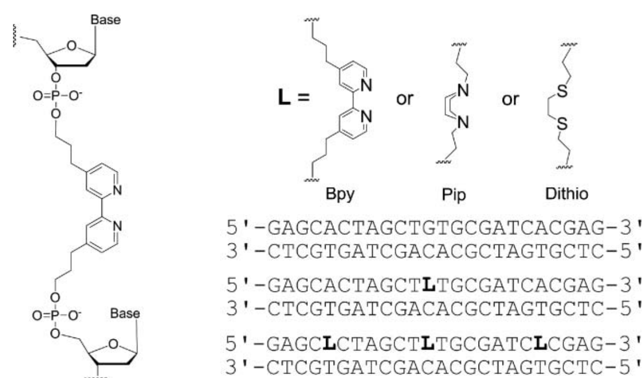

Fig. 1 (a) Illustration of a 2,2′-bipyridine nucleoside incorporated into DNA. (b) Control and ligand-modified ODN sequences with ligand positions designated by L.

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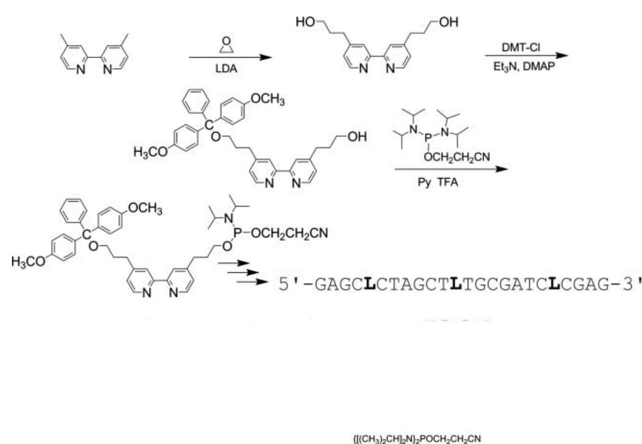

Fig. 2 Synthesis of the Bpy-containing oligodeoxyribonucleotides.

was replaced with a ligand and one in which three nucleosides at positions 5, 12 and 19 were replaced (Fig. 1). Control ODNs without ligands are also shown in Fig. 1.

The ODNs of Fig. 1 were combined with the appropriate complements. Duplex stabilities were then determined by melting temperature (T_m) measurements (*cf.*, Fig. 3) in which single stranded DNA displays higher absorbance at 260 nm than double stranded. Monitoring A_{260} as a function of temperature shows how strongly two strands interact. Higher T_m's indicate greater stability. Both melting and annealing curves were determined over an 80 °C temperature range and were superimposable. Melting temperatures were determined for ligand : metal ratios of 1 : 1 and 1 : 10 using a variety of different metal ions. The ten metal ions examined were Cu^{1+}, Cu^{2+}, Mg^{2+}, Fe^{2+}, Pd^{2+} (from $PdCl_4^{2-}$ treated with 3.8 equiv. $AgNO_3$), Pd^{4+} (from $PdCl_6^{2-}$ treated with 5.8 equiv. $AgNO_3$), Zn^{2+}, Pt^{2+} (from $PtCl_4^{2-}$ treated with 3.8 equiv. $AgNO_3$), Pt^{4+} (from $PtCl_6^{2-}$ treated with 5.8. equiv. $AgNO_3$) and Os^{4+} (from $OsCl_6^{2-}$ treated with 5.8 equiv. $AgNO_3$).

Incorporation of a single Bpy ligand into the ODN provided a destabilizing effect, evidenced by a T_m ~13 °C lower than a corresponding unmodified ODN (Table 1). Addition of one equiv. Pt^{2+}, Pt^{4+}, Pd^{4+} or Os^{4+} stabilized the duplex and raised the T_m between ~6 and 11 °C (Fig. 3(a), Table 1). Addition of Cu^{2+}, Mg^{2+}, Fe^{2+}, Pd^{2+} or Zn^{2+} had little effect on the stability of the 1 × Bpy duplex. The T_m of the Bpy-modified ODN and unmodified complement with 1 equiv. Os^{4+} (T_m = 65.1 °C) or Pt^{2+} (T_m = 64.3 °C) was similar to that of the unmodified ODN (T_m = 67.5 °C). Thus, the duplex stability, which had been decreased by the inserted Bpy ligand, was restored in the presence

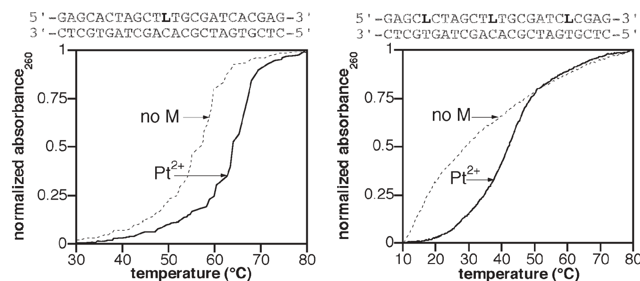

Fig. 3 Melting curves of Bpy-containing ODNs with and without 1 equiv. Pt^{2+} per ligand: (a) 1 × Bpy ODN, (b) 3 × Bpy ODN.

Table 1 Melting temperatures (°C) of duplexes[a]

oligo	No M	Pt^{2+}	Pt^{4+}	Pd^{4+}	Cu^{2+}	Os^{4+}
Control	67.5	67.5	68.4	67.6	68.5	67.9
1 × Bpy	54.1	64.3	61.2	60.3	56.4	65.1
3 × Bpy	—	36.0	39.0	41.7	29.4	38.8

[a] Measured in 50 mM tris buffer, 50 mM NaCl, pH 7.4, 1 μM per strand and 1 equiv. metal per ligand.

of these ions. By contrast, metal salts did not influence the stability of the unmodified duplex controls (Table 1).

Incorporation of three ligands into the ODN strands destabilized the duplex, such that no annealing occurred, as was noted by the absence of a sigmoidal melting curve (Fig. 3(b), Table 1). Adding one equiv. Cu^{2+}, Pd^{4+}, Pt^{2+}, Pt^{4+} or Os^{4+} per ligand recovered normal duplex formation, with a T_m in the range of ~30–40 °C observed (Fig. 3(b), Table 1). No stabilizing effect was observed for the other metal ions assayed. This stabilization was not intensified with excess metal ions at 10 equiv. per ligand. Increased T_m's of metal-chelating ODNs in the presence of one equiv. of various transition metal ions, coupled with no T_m changes for unmodified ODNs, suggest metal binding at the ligands.

Both the Pip and Dithio-containing ODNs also demonstrated enhanced stability with added metal ions, although to a lesser extent than the Bpy ODNs. For the single nucleoside replacement duplex containing piperazine (Fig. 1), the starting T_m was ~14 °C lower (T_m = 53.9 °C) than that of the unmodified oligodeoxyribonucleotide (T_m = 67.5 °C). Addition of Pt^{2+} and Pd^{4+} did not affect the T_m significantly. However, with three Pip replacements in an ODN strand, a more dramatic effect was observed. Alone, the 3 × Pip ODN exhibited no duplex properties. Addition of 10 equiv. Pt^{2+} (T_m = 35.4 °C) or Pd^{4+} (T_m = 32.3 °C) per ligand brought about duplex formation; however, the effect of only 1 equiv. metal per ligand was minimal. For the Dithio-containing ODNs, a similar trend was observed. Again, the presence of one Dithio ligand destabilized the duplex (T_m = 53.2 °C) relative to the unmodified oligodeoxyribonucleotides. Three Dithio nucleoside replacements were required to observe enhanced stability upon metal addition. No annealing of the 3 × Dithio ODN was observed without metals but a duplex was restored with 10 equiv. Pt^{2+} (T_m = 32.3 °C), Pd^{4+} (T_m = 34.6 °C), or Os^{4+} (T_m = 36.3 °C) per ligand. The other ions mentioned above did not yield a detectable melting curve.

These modified ODNs may be viewed as inorganic analogues of prior studies in which hydrophobic groups have been added to DNA.[48–50] Incorporation of cholesterol and long alkyl chains into ODNs can alter duplex stability, influence cellular uptake, and decrease nuclease susceptibility.[48–50] An earlier report of placing bpy into the DNA backbone showed formation of substitutionally inert $Ru(bpy)_3$ complexes tethered inside the ODN with oligo(ethylene glycol) chains.[30] In our work we have located the bpy closer to the DNA backbone and not "covered" the metal center with additional large ligands. The resulting metal complexes are thus smaller and open to interaction with the complimentary strand.

Here we have designed and synthesized a new class of metal-chelating nucleic acids in which ligands are incorporated into the backbone. As demonstrated by the UV data, these

ligand-containing ODNs bind metal ions and generate stable DNA duplexes with complementary ODNs in the presence of certain metal ions. Control ODNs demonstrated no enhancement of stability upon metal ion addition. Stabilization of these duplexes may be a result of directing the bonding of cationic metal ions into the otherwise anionic nucleic acid environment. Alternatively, hydrogen bonding with or coordination of metal ions by complementary bases may be at play. The variety of T_m's found here for ligand-modified ODN duplexes with different metal ions suggests the future ability to have fine control over duplex stability. We may be able to use such nucleic acids for targeting specific genes or form duplexes no longer constrained by the standard base pairing motifs.

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Notes and references

1 T. D. Ros, G. Spalluto, M. Prato, T. Saison-Behmoaras, A. Boutorine and B. Cacciari, *Curr. Med. Chem.*, 2005, **12**, 71.
2 H. Yan, *Science*, 2004, **306**, 2048.
3 J. Wang, *Anal. Chim. Acta*, 2002, **469**, 63.
4 N. Seeman, *Trends Biochem. Sci.*, 2005, **30**, 119.
5 K. J. Scanlon, *Curr. Pharm. Biotechnol.*, 2005, **5**, 415.
6 J. Kurreck, *Eur. J. Biochem.*, 2003, **270**, 1628.
7 P. Wittung, P. E. Nielsen, O. Buchardt, M. Egholm and B. Norden, *Nature*, 1994, **368**, 561.
8 S. K. Singh, P. Neilsen, A. A. Koshkin and J. Wengel, *Chem. Commun.*, 1998, 455.
9 M. Matsukura, K. Shinozuka, G. Zon, J. Mitsuya, M. Reitz, J. S. Cohen and S. Broder, *Proc. Natl. Acad. Sci. USA*, 1987, **84**, 7706.
10 J. Summerton, *Biochim. Biophys. Acta*, 1999, **1489**, 141.
11 Z. J. Lesnikowski, *Curr. Org. Chem.*, 2007, **11**, 355.
12 J. Müller, *Nature*, 2006, **444**, 698.
13 K. Tanaka, G. H. Clever, Y. Takezawa, Y. Yamada, C. Kaul, M. Shionoya and T. Carell, *Nat. Nanotechnol.*, 2006, **1**, 190.
14 K. Tanaka, A. Tengeiji, T. Kato, N. Toyama and M. Shionoya, *Science*, 2003, **299**, 1212.
15 L. Cai, K. Lim, S. Ren, R. S. Cadena and W. T. Beck, *J. Med. Chem.*, 2001, **44**, 2959.
16 D. J. Hurley and Y. Tor, *J. Am. Chem. Soc.*, 1998, **120**, 2194.
17 E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg and P. G. Shultz, *J. Am. Chem. Soc.*, 2000, **122**, 10714.
18 L. Zhang and E. Meggers, *J. Am. Chem. Soc.*, 2005, **127**, 74.
19 R. M. Watson, Y. A. Skorik, G. K. Patra and C. Achim, *J. Am. Chem. Soc.*, 2005, **127**, 14628.
20 M. Shionoya and K. Tanaka, *Curr. Opin. Chem. Biol.*, 2004, **8**, 592.
21 J. Morales-Rojas and E. T. Kool, *Org. Lett.*, 2002, **4**, 4377.
22 S. Ren, C. Lisheng and B. M. Segal, *J. Chem. Soc., Dalton Trans.*, 1991, 1413.
23 N. L. Frank and T. J. Meade, *Inorg. Chem.*, 2003, **42**, 1039.
24 K. V. Gothelf, A. Thomsen, M. Nielsen, E. Clo and R. S. Brown, *J. Am. Chem. Soc.*, 2004, **126**, 1044.
25 J. Hovinen and H. Hakala, *Org. Lett.*, 2001, **3**, 2473.
26 M. Göritz and R. Krämer, *J. Am. Chem. Soc.*, 2005, **127**, 18016.
27 J. J. Rack, E. S. Krider and T. J. Meade, *J. Am. Chem. Soc.*, 2000, **122**, 6287.
28 E. S. Krider, J. E. Miller and T. J. Meade, *Bioconjugate Chem.*, 2002, **13**, 155.
29 G. Wang, J. Yuan, K. Matsumoto and Z. Hu, *Anal. Biochem.*, 2001, **299**, 169.
30 K. Wiederholt and L. W. McLaughlin, *Nucleic Acids Res.*, 1999, **27**, 2487.
31 A. Galeone, L. Mayol, G. Oliviero, D. Rigano and M. Varra, *Bioorg. Med. Chem. Lett.*, 2001, **11**, 383.
32 J. L. Czlapinski and T. L. Sheppard, *J. Am. Chem. Soc.*, 2001, **123**, 8618.
33 C. Price, B. R. Horrocks, A. Mayeux, M. R. J. Elsegood, W. Clegg and A. Houlton, *Angew. Chem., Int. Ed.*, 2002, **41**, 1047.
34 F. D. Lewis, S. A. Helvoigt and R. L. Letsinger, *Chem. Commun.*, 1999, 327.
35 G. Bianké, V. Chaurin, M. Egorov, J. Lebreton, E. C. Constable, C. E. Housecroft and R. Haner, *Bioconjugate Chem.*, 2006, **17**, 1441.
36 Z.-H. Liu, J.-J. Zhang and W.-J. Zhang, *Inorg. Chim. Acta*, 2006, **359**, 519.
37 M. Beller, H. Trauthwein, M. Eichberger, C. Breindl, T. E. Müller and A. Zapf, *J. Organomet. Chem.*, 1998, **566**, 277.
38 O. Hassel and B. F. Pedersen, *Proc. Chem. Soc. London*, 1959, 394.
39 A. Marzotto, D. A. Clemente and G. Valle, *Acta Crystallogr., Sect. C*, 1997, **53**, 1580.
40 M. M. Olmstead, W. K. Musker, L. W. Ter Haar and W. E. Hatfield, *J. Am. Chem. Soc.*, 1982, 104.
41 F. A. Cotton and D. L. Weaver, *J. Am. Chem. Soc.*, 1965, **87**, 4189.
42 W. K. Musker, M. M. Olmstead, R. M. Kessler, M. B. Murphey, C. H. Neagley, P. B. Roush, N. L. Hill, T. L. Wolford, H. Hope, G. Delker, K. Swanson and B. V. Gorewit, *J. Am. Chem. Soc.*, 1980, **102**, 1225.
43 E. W. Abel, I. Moss, K. G. Orrell, V. Sik, D. Stephenson, P. A. Bates and M. B. Hursthouse, *J. Chem. Soc., Dalton Trans.*, 1988, 521.
44 E. Linder, R. Veigel, K. Ortner, C. Natchigal and M. Steimann, *Eur. J. Inorg. Chem.*, 2000, 959.
45 R. Faithi, J. Rudolph, R. G. Gentles, R. Patel, E. W. Macmillan, M. S. Reitman, D. Pelham and A. F. Cook, *J. Org. Chem.*, 1996, **61**, 5600.
46 Y. S. Sanghvi, Z. Guo, H. M. Pfundheller and A. Converso, *Org. Process Res. Dev.*, 2000, **4**, 175.
47 K. Miyoshi, T. Miyake, T. Hozumi and K. Itakura, *Nucleic Acids Res.*, 1980, **8**, 5473.
48 C. MacKellar, D. Graham, D. W. Will, S. Burgess and T. Brown, *Nucleic Acids Res.*, 1992, **20**, 3411.
49 R. L. Letsinger, S. K. Chaturvedi, F. Farooqui and M. Salunkhe, *J. Am. Chem. Soc.*, 1993, **115**, 7535.
50 S. M. Gryaznov and D. H. Lloyd, *Nucleic Acids Res.*, 1993, **21**, 5909.

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