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Enhanced cooperative binding of oligonucleotides to form DNA duplexes mediated by metal ion chelation†

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Received (in Cambridge, UK) 26th June 2002, Accepted 19th July 2002
First published as an Advance Article on the web 5th August 2002

Metal chelating iminodiacetic acid moieties were appended to abutting ends of two 9-mer oligonucleotides designed to hybridize contiguously on an 18-mer target. Chelation of 1 equivalent of Gd^{3+} cooperatively increases affinities of the oligonucleotides to the single stranded DNA target as evidenced by a 15 °C increase in T_m of the complex.

There is significant interest in new chemical strategies to enhance the binding of oligonucleotides to nucleic acid targets. One driving force is that single-stranded short oligonucleotides possess attractive features for therapeutic applications,[1,2] but their affinities for the nucleic acid target are low. Strategies to enhance their affinity include using intercalators,[3] positive charges,[4] modified nucleobases or nucleosides[5] or introducing cooperative interactions between two oligonucleotides[6,7] that bind to the same target molecule. Interstrand metal chelation between complementary unnatural base pairs has been used to enhance duplex stability.[8] In this paper we report the use of metal ion chelation between two short oligonucleotides that assemble on a single stranded DNA target leading to enhanced binding.

The strategy has employed metal chelation at the ends of two oligonucleotides hybridized to the same target (Fig. 1). Two 9-mer oligodeoxyribonucleotides $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$ (Fig. 2) were designed to bind to adjacent sites on the 18-mer oligonucleotide, $\mathbf{C_0}$ with predicted free energies of −8.8 kcal mol^{-1} and −9.1 kcal mol^{-1}, respectively.[9]

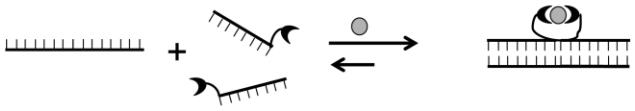


Fig. 1 Metal chelation to enhance oligonucleotide binding.

Iminodiacetic acid (IDA), forms stable bis-chelates with several metal ions.[10] However, Gd^{3+} was chosen since the free energy of formation of a 2:1 complex of IDA and Gd^{3+} (−7.3 kcal mol^{-1})[11] is relatively close to the associated hybridization energies of $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$. Amino-modified oligonucleotides $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$ were appended *via* an amide linkage to IDA moieties to give $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ respectively.‡ UV melting profiles§ were measured for various combinations of oligonucleotides and Gd^{3+}, with the target $\mathbf{C_0}$ (Table 1) which afforded the melting temperature, T_m.[12] The amino-modified oligonucleotides $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$, individually bound to $\mathbf{C_0}$, gave comparable T_m values that were in good agreement with predicted values (Table 1).[9] The simultaneous binding of $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$ to $\mathbf{C_0}$ resulted in a 2.7 ± 1 °C increase in T_m (Table 1), indicating a small cooperative effect possibly due to π-interactions of the terminal bases.[6b] Addition of Gd^{3+} to the ternary complex of $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$ bound to $\mathbf{C_0}$ showed no change in T_m, suggesting that unchelated Gd^{3+} did not have any effect on duplex stability. When the IDA modified analogues, $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ were each individually bound to $\mathbf{C_0}$ (Table 1), there was a 3.7 ± 1 °C decrease in the T_m value as compared to $\mathbf{A_{AM}}$ and $\mathbf{B_{AM}}$. This may be an electrostatic consequence of replacing the positively charged amino group by a negatively charged carboxylate. However, the simultaneous binding of both $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ to $\mathbf{C_0}$ showed a 3.2 ± 1 °C increase in T_m (Table 1) indicating some cooperativity, contrary to what might be expected on the basis of electrostatic interactions alone.

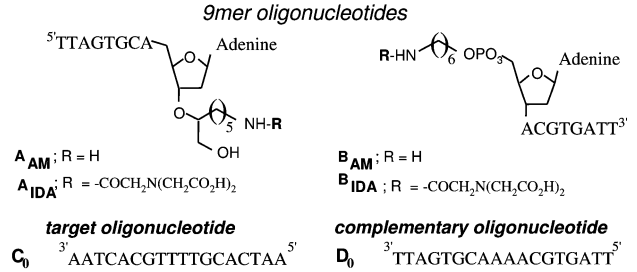


Fig. 2 Oligonucleotides used in this study.

The simultaneous binding of $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ to $\mathbf{C_0}$ was investigated in the presence of 1 mol equivalent of Gd^{3+} (2 μM) with various incubation times (*t*). The melting curve showed two transitions shown in the first derivative plot of absorbance (*A*) *vs.* temperature (*T*) (Fig. 3A). The first transition corresponds to a species melting at 28 °C, consistent with unmetallated species based on the control experiment lacking Gd^{3+} (Table 1). The second transition indicates a higher stability species melting at 43 °C, consistent with metallated species (Table 1). It was apparent that in the presence of 1 mol equiv. Gd^{3+}, the proportion of high T_m species increased with time (Fig. 3A) at the expense of the low T_m species. That 1 mol equiv. Gd^{3+} (2 μM) resulted in ~80% conversion to the high T_m species at t = 30 h (Fig. 3B) suggests a Gd^{3+} stoichiometry of one in the metallated DNA duplex. The addition of excess Gd^{3+} (40 μM) to the complex of $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ bound to $\mathbf{C_0}$ resulted in the high T_m species being formed exclusively. Fig. 3B shows the melting curves of $\mathbf{A_{IDA}}$ and $\mathbf{B_{IDA}}$ bound to $\mathbf{C_0}$ without Gd^{3+}

Table 1 T_m values calculated from UV melting analysis of IDA-modified and unmodified 9-mers binding to the target 18-mer under various conditions

Oligonucleotides	$[Gd^{3+}]$/μM	T_m[a]/°C
$\mathbf{A_{AM}}$, $\mathbf{C_0}$	0	28.9 (28.2)
$\mathbf{B_{AM}}$, $\mathbf{C_0}$	0	28.7 (29.4)
$\mathbf{A_{AM}}$,$\mathbf{B_{AM}}$, $\mathbf{C_0}$	0	31.5
$\mathbf{A_{AM}}$,$\mathbf{B_{AM}}$, $\mathbf{C_0}$	40[b]	30.5
$\mathbf{A_{IDA}}$, $\mathbf{C_0}$	0	25.3
$\mathbf{B_{IDA}}$, $\mathbf{C_0}$	0	24.8
$\mathbf{A_{IDA}}$, $\mathbf{C_0}$	40[b]	25.2
$\mathbf{B_{IDA}}$, $\mathbf{C_0}$	40[b]	24.2
$\mathbf{A_{IDA}}$,$\mathbf{B_{IDA}}$, $\mathbf{C_0}$	0	28.3
$\mathbf{A_{IDA}}$,$\mathbf{B_{IDA}}$, $\mathbf{C_0}$	2[c]	27.5, 42.6[d]
$\mathbf{A_{IDA}}$,$\mathbf{B_{IDA}}$, $\mathbf{C_0}$	40[b]	39.6
$\mathbf{D_0}$, $\mathbf{C_0}$	0	59.4 (56.4)

[a] Associated errors are ± 1 °C, T_m values predicted by nearest neighbour analysis are in parantheses;[10] Incubation time. [b] 30 h. [c] 5 min. [d] Dual transitions were obtained.

† Electronic supplementary information (ESI) available: experimental details. See http://www.rsc.org/suppdata/cc/b2/b206054j/

DOI: 10.1039/b206054j

Table 2 Values of connection Gibbs energy (ΔG_S) and cooperative energy of interaction (E_{coop}) calculated from UV melting study data

Interacting components	[Gd^{3+}]/ μM	K_1[a] /l mol^{-1}	K_2[a] /l mol^{-1}	K_{1+2}/l^2 mol^{-2}	$K_{1,2}$[b] /l mol^{-1}	$K_{2,1}$[b] /l mol^{-1}	ΔG_s[c]/ kcal mol^{-1}	E_{coop}[d]/ kcal mol^{-1}
A_{AM}, B_{AM}, C_o	0	3.08×10^6	2.44×10^6	2.34×10^{13}	9.59×10^6	7.60×10^6	−0.67	−0.74
A_{IDA}, B_{IDA}, C_o	0	1.14×10^6	9.52×10^5	8.40×10^{12}	8.82×10^6	7.37×10^6	−0.93	−1.42
A_{IDA}, B_{IDA}, C_o	40[e]	1.21×10^6	9.43×10^5	4.57×10^{15}	4.85×10^9	3.78×10^9	−4.91	−5.14

[a] K_1 and K_2 = equilibrium constant for binding of **A_{AM}** (or **A_{IDA}**) and **B_{AM}** (or **B_{IDA}**) to **C_0** at 298 K. [b] $K_{1,2}$ = equilibrium constant for the binding of **A_{AM}** (or **A_{IDA}**) on the binary complex of **B_{AM}** (or **B_{IDA}**) and **C_0** at 298 K; $K_{2,1}$ = equilibrium constant for the binding of **B_{AM}** (or **B_{IDA}**) on the binary complex of **A_{AM}** (or **A_{IDA}**) and **C_0** at 298 K; $K_{1+2} = K_1K_{2,1} = K_2K_{2,1}$. [c] ΔG_S was calculated using the Jencks' model (errors are ± 4%).[14] [d] E_{coop} was calculated using the Hill model (errors are ± 1–3%).[15] [e] Incubation time = 30 h.

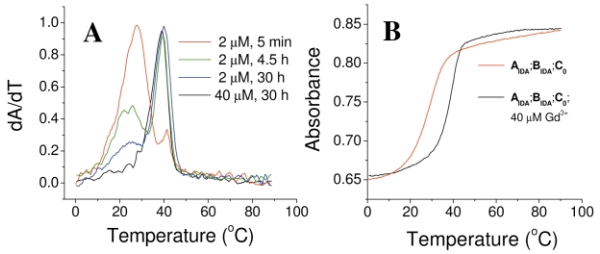
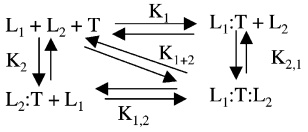


Fig. 3 A, First derivative plots of absorbance (*A*) *vs.* temperature (*T*) from the melting curves of **A_{IDA}** and **B_{IDA}** bound to **C_0** in the presence of varying Gd^{3+} concentrations and incubation times. B, Melting curves of **A_{IDA}** and **B_{IDA}** bound to **C_0** in the absence and presence of Gd^{3+}

and in the presence of 40 μM Gd^{3+} (t = 30 h). In the presence of Gd^{3+} a significant T_m enhancement of 15 °C was observed, with an associated steepening of the melting curve indicating a more cooperative transition in the presence of the metal. An evaluation of the cooperative enhancement of binding due to metallation, allows comparisons with other systems. Equilibrium constants and associated thermodynamic parameters at 298 K were calculated from the UV melting curves assuming a two-state (all-or-none) model for the melting transition.[13] Cooperative enhancement was evaluated from the associated thermodynamic parameters for the ternary complex in the cooperative mode using the Jencks[14] and the Hill[15] models, both of which have been widely used in comparable studies.

The formation of a ternary complex, L_1:T:L_2, from ligands L_1 and L_2 binding to T may be represented by the equilibrium in Scheme 1 with corresponding association constants.[6c] K_{1+2} represents the overall binding equilibrium between the fully assembled complex and its individual components. The values of K_1, K_2 and K_{1+2} were calculated for the binding of **A_{AM}** or **B_{AM}** to **C_0**, **A_{IDA}** or **B_{IDA}** to **C_0** in the absence and presence of Gd^{3+} (Table 1). The energy corresponding to the cooperative effect expressed as either ΔG_s (the Gibbs connection energy)[14] or E_{coop} (the Gibbs free energy change for cooperative enhancement)[15] were calculated for the binding of **A_{IDA}** and **B_{IDA}** to **C_0** in the presence and absence of Gd^{3+} (Table 2).



Scheme 1 Equillibrium for ternary complex formation with associated equilibrium constants.[16]

The simultaneous binding of **A_{AM}** and **B_{AM}** to **C_0** afforded an E_{coop} of −0.74 kcal mol^{-1}. Binding of unmetallated ligands **A_{IDA}** and **B_{IDA}** to **C_0** furnished an E_{coop} value of −1.42 kcal mol^{-1}. However, in the presence of 40 μM Gd^{3+}, binding of **A_{IDA}** and **B_{IDA}** to **C_0**, with an associated ΔG_s of −4.91 kcal mol^{-1} and an E_{coop} of −5.14 kcal mol^{-1} (Table 2). The cooperative enhancement obtained for the present system is favourable in comparison to several studies employing other cooperative approaches for the binding of short (9–12 mer) oligonucleotides to single stranded DNA, where typical E_{coop} values range between −0.7 to −2.1 kcal mol^{-1} or show T_m enhancements of ~10 °C.[6a,d,7] Thus metal ion chelation can generate significant enhancement of duplex stability. This paves the way for targeting relatively long sequences of single stranded nucleic acids using shorter oligonucleotide segments under the control of a metal ion switch.

We thank the BBSRC for a studentship to I. S. and Dudley Williams for valuable discussions.

Notes and references

‡ The synthetic route to **A_{IDA}** and **B_{IDA}** was based on a protocol described by Endo and Komiyama.[17] MW (obtained from negative ion ESI-MS): **A_{IDA}**: 3121 (calc.: 3118); **B_{IDA}**: 3091 (calc.: 3087).

§ T_m studies were conducted on a Varian-Carey 1E UV-visible spectrophotometer at 260 nm. Each oligonucleotide sample (2 μM, 0.4 mL) in buffer (10 mM K_3PO_4, pH 7.0, 0.15 M NaCl) was annealed (heated to 90 °C and cooled to 0 °C over 1.5 h) prior to measurements (0–90 °C at a rate of 1°C min^{-1}). Gd^{3+} was added in a volume of 4 μL. Data processing was carried out on Microcal Origin 4.1 software.

1 S. L. Loke, C. A. Stein, S. H. Zhang, K. Mori, M. Nakanishi, C. Subhasinghe, J. S. Cohen and L. M. Neckers, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 3474–3478.
2 E. T. Kool, *Chem. Rev.*, 1997, **97**, 1473–1487.
3 J. S. Sun, J. C. Francois, T. Montenay-Garestior, T. Saison-Behmoaras, V. Roig, N. T. Thuong and C. Hélène, *Proc. Natl. Acad. Sci. USA*, 1989, **86**, 9198–9202.
4 (*a*) J. G. Harrison and S. Balasubramanian, *Nucleic Acids Res.*, 1998, **26**, 3136–3145; (*b*) P. Dande, G. Liang, F. X. Chen, C. Roberts, M. G. Nelson, H. Hashimoto, C. Switzer and B. Gold, *Biochemistry*, 1997, **36**, 6024–6032.
5 (*a*) K-Y. Lin and M. D. Matteucci, *J. Am. Chem. Soc.*, 1998, **120**, 8531–8532; (*b*) S. Obika, T. Uneda, T. Sugimoto, D. Nanbu, T. Minami, T. Doi and T. Imanishi, *Bioorg. Med. Chem. Lett.*, 2001, **9**, 1001–1011; (*c*) R. Kumar, S. K. Singh, V. K. Rajwanshi, M. Meldgaard and J. Wengel, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2219–2222.
6 (*a*) S. M. Gryaznov and D. H. Lloyd, *Nucleic Acids Res.*, 1993, **21**, 5909–5915; (*b*) S. A. Strobel and P. B. Dervan, *J. Am. Chem. Soc.*, 1989, **111**, 7286–7287; (*c*) N. Colocci, M. D. Distefano and P. B. Dervan, *J. Am. Chem. Soc.*, 1993, **115**, 4468–4473; (*d*) E. R. Kandimalla, A. Manning, C. Lathan, R. A. Byrn and S. Agrawal, *Nucleic Acids Res.*, 1995, **23**, 3578–3584.
7 Metal chelation has been employed to enhance stability of triplexes: (*a*) T. Ihara, Y. Takeda and A. Jyo, *J. Am. Chem. Soc.*, 2001, **123**, 1772–1773; (*b*) S. Sueda, T. Ihara and M. Takagi, *Chem. Lett.*, 1997, **11**, 1085–1086.
8 E. Meggers, P. L. Holland, W. B. Tolman, F. E. Romesberg and P. G. Schultz, *J. Am. Chem. Soc.*, 2000, **122**, 10714–10715.
9 J. J. SantaLucia, H. T. Allawi and P. A. Seneviratne, *Biochemistry*, 1996, **35**, 3555–3562.
10 D. D. Perrin, in *Stability Constants of Metal-ion Complexes: Part B, Organic Ligands*, Pergamon Press, Oxford, 1979, pp. 211–220.
11 L. Gunnar Sillen and A. E. Martell, *Stability constants of Metal-Ligand Complexes; No1*, The Chemical Society, London, 1971, pp. 339–341.
12 D. Porshcke, *Biopolymers*, 1971, **10**, 1989–2013.
13 L. A. Marky and K. J. Breslauer, *Biopolymers*, 1987, **26**, 1601–1620.
14 W. P. Jencks, *Proc. Natl. Acad. Sci. USA*, 1981, **78**, 4046–4050.
15 T. L. Hill, *Cooperativity Theory in Biochemistry: Steady State and Equilibrium Systems*, Springer-Verlag, New York, 1985.
16 The equilibrium assumes that (a) ligands L_1 and L_2 do not interact and (b) do not compete for each other's binding sites on T.
17 M. Endo and M. Komiyama, *J. Org. Chem.*, 1996, **61**, 1994–2000.