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Peptide Nucleic Acid–Metal Complex Conjugates: Facile Modulation of PNA–DNA Duplex Stability

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Abstract—Conjugates of peptide nucleic acids (PNA) and metal binding ligands were prepared using solid-phase synthesis. Stability of duplexes of bis-picolylamine–PNA conjugates and DNA was found to be modulated by equimolar concentrations of bioavailable metal ions: Ni^{2+}, $Zn^{2+} > Cu^{2+}$. Sequence specificity of PNA was not compromised in the presence of these metal ions.

Peptide nucleic acids (PNAs) are DNA analogues, in which the sugar-phosphate backbone has been substituted by *N*-(2-aminoethyl)-glycine units. Apart from high chemical stability and nucleic acids binding affinity and specificity, PNA chemistry is simple and straightforward.[1] Applications of PNA for suppression of gene expression in vivo are emerging.[2] We are interested in the control of PNA binding to oligonucleotide targets by bioavailable metal ions. Since intracellular concentration of unbound transition metals is dependent on cell type,[3] metal-responsive PNA–DNA/RNA binding can be employed for selective suppression of gene expression in these specific cells. Strategies for metal dependent duplex formation, which are reported up to date, include intrastrand metal binding between non-natural basepairs[4] and cooperative metal binding between two terminally modified DNA.[5] In the former method both the probe and the target DNA should be modified, while in the latter, two probes, which are modified at either 5′- or 3′-ends, are necessary. In this paper, we report on an approach (Fig. 1) requiring only one terminally modified PNA-probe, natural DNA target and importantly Zn^{II} ions, which is the most abundant transition metal in the cell.[6] In contrast to the reported methods, our approach is potentially suitable for in vivo experiments.

We have chosen to covalently attach to the N-terminus of PNA bi- and tri-dentate ligands (Scheme 1) capable of forming 1:1 complexes with Zn^{II}, Cu^{II} and Ni^{II}. 1:1 Metal complexes with these ligands have free coordination sites, which can enable direct coordinative interaction of metal ion with, for example, N-atoms of DNA nucleosides or O-atoms of phosphodiester groups of the DNA backbone. The ligands were attached to PNA via linkers of different length for optimization of metal complex–DNA interaction (Scheme 1). Synthesis of conjugates **5**, **6** (Scheme 1) was accomplished using sequential coupling/deprotection steps of a required number of Fmoc-Gly-OH building blocks onto the terminal amino-group of otherwise fully protected and Rink-resin bound PNA **2**[7] to give **3**, then final acylation with 6-(2-pyridyl)-4-pyrimidine carboxylic acid ($\mathbf{L^1}$-CO_2H, **4**), and standard PNA deprotection and cleavage using TFA/*m*-cresol mixture (**5**, **6**).[8] Conjugates **7**, **8** were synthesized analogously except 5-(2-pyridyl)-1H-3-pyrazole carboxylic acid ($\mathbf{L^2}$-CO_2H) was used in the last acylation step. Synthesis of conjugates **10** and **11** was performed by acylation of **3** with bromoacetyl bromide to give **9**, amination with bis-(2-picolyl)-amine ($\mathbf{L^3}$)[9] and final PNA deprotection and cleavage. Purity of crude products was 60–75% according to MALDI-TOF mass spectrometric and HPLC analysis. All PNA conjugates were HPLC purified.[10] Fractions containing more than 90% of desired product were combined, lyophilized, dissolved in deionized water and used for further experiments.[11] The molecular ions of metal complexes of conjugates **5**–**8** and non-modified **12** are not detectable in MALDI-TOF mass spectra of mixtures of the PNA (2 μM) with the metal salts (1–50 equiv), whereas mass spectra of equimolar mixtures of conjugates **10**, **11**, and Ni^{II} or Cu^{II} contain predominant peaks corresponding

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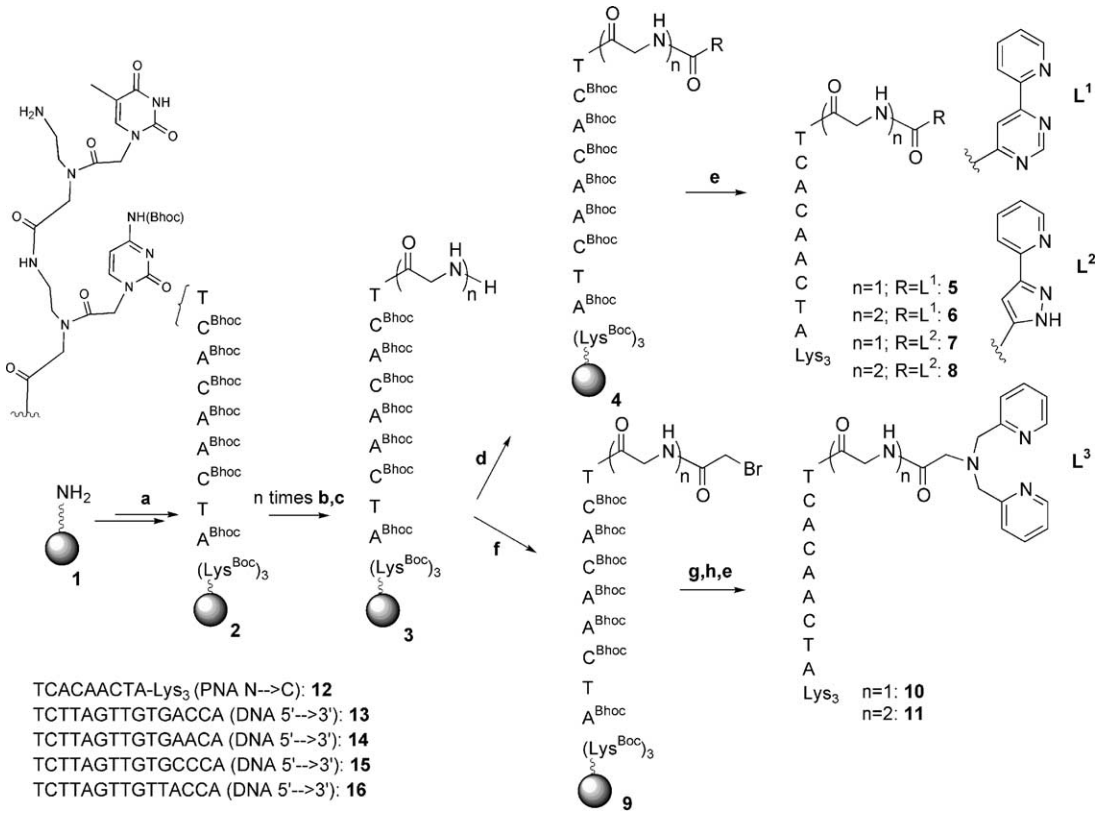

Scheme 1. Synthesis of PNA, which are N-terminally modified with metal binding ligands. Balls represent polymeric support. Reaction conditions: (a) PNA synthesis;[12] (b) Fmoc-Gly-OH, HBTU, HOBT, DIEA, DMF; (c) piperidine, DMF; (d) RCO_2H, HBTU, HOBT, DIEA, DMF; (e) TFA, *m*-cresol; (g) bromoacetylbromide, DIEA, DMF; (h) di-(2-picolyl)amine, DIEA, DMF.

to metal complexes with PNA. Zn^{II}-complexes of all conjugates are not detectable in MALDI-TOF mass spectra. Fluorescent indicators of Zn^{II} and Ni^{II} (Newport Green[12]) and Cu^{II} (Calcein[12]) do not detect unbound Zn^{II}, Ni^{II} and Cu^{II} ions in equimolar (2 μM) solutions of metals and **10** or **11**. In contrast, fluorescence of the metal indicators is practically identical in solutions of free metals and metals with **5**, **6**, **7**, **8** or **12** indicating insignificant binding of metals to **5**–**8**, **12** PNA.

Melting points of **5**–**8** PNA–DNA duplexes are similar both in the absence and in the presence of metal ions (Table 1), while duplexes **10**–**11**:**13** are melted 2.6–7.0 °C higher in the presence of M^{II} (Table 1). Stability constants (logK) of ca. 14, 9 and 7.6 have been reported for the Cu^{II}, Ni^{II} and Zn^{II} complexes of di-2-picolylamine, $\mathbf{L^3}$ (pK_a of $\mathbf{HL^3}$ is 7.1).[13] Interestingly, hysteresis is observed in the melting experiment with [Zn^{II}(**10**)] when overall Zn^{II} concentration is 2 μM. Obviously, dissociation of the least stable complex [Zn^{II}(**10**)] is already significant, especially at high temperature, and reformation of [Zn^{II}(**10**)] at cooling is slow in highly dilute solution. Dissociation of [Zn^{II}(**10**)] is suppressed by the use of excess Zn^{II} (4 μM, Table 1). The sequence specificity of the selected metal complex conjugates (entries 2, 3) was found to be comparable to that of the non-modified PNA (entry 4)[14] (Table 2), indicating that

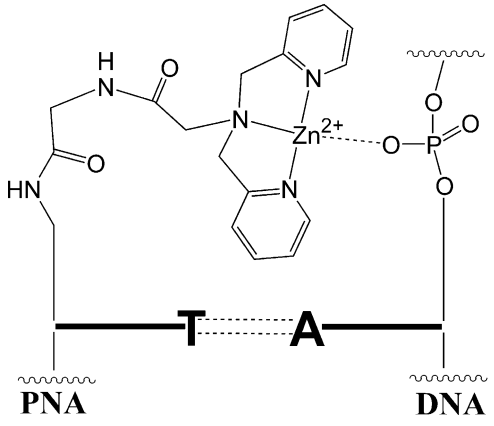

Figure 1. A proposed approach for metal-dependent binding of PNA probes to oligonucleotide targets.

Table 1. UV melting points of PNA-DNA duplexes[a]

Duplex	T_m, °C			
	No metal	Zn^{II} 4 μM	Cu^{II} 2 μM	Ni^{II} 2 μM
5:**13**	59.0±0.7	58.9±0.8	57.8±2.5	58.5±1.2
6:**13**	58.7±1.1	59.2±2.1	59.0±1.8	59.1±1.9
7:**13**	59.1±1.2	58.7±2.2	58.1±2.5	58.9±1.4
8:**13**	58.1±1.9	59.1±1.6	58.9±1.0	59.3±2.1
10:**13**	57.2±0.8	63.4±1.1	60.2±1.2	64.2±0.2
11:**13**	57.7±1.8	63.4±0.6	60.3±1.0	61.5±2.5
12:**13**	61.4±0.8	60.3±1.2	59.6±0.7	61.5±0.7

[a]Average of at least four melting points±SD at 2 μM PNA and DNA strand concentration, MOPS pH 7 10 mM, NaCl 50 mM. After mixing all components, the mixtures were pre-equilibrated before T_m-experiments by heating to 90 °C and cooling (2 °C/min) to 22 °C.

Table 2. Mismatch discrimination experiments[a]

PNA[b]	Target DNA			
	13	**14**	**15**	**16**
10	57.2±0.8	57.2±1.6	59.8±1.8	47.4±0.1
10/Zn^{II}	63.4±1.1	62.8±0.8	63.9±2.2	57.4±0.5
10/Cu^{II}	60.2±1.2	60.2±2.3	58.2±3.8	53.0±1.6
12	61.4±0.8	59.2±1.4	60.2±1.7	52.9±2.3

[a]See footnote of Table 1.
[b]$[Zn^{II}]=4$ μM, $[Cu^{II}]=2$ μM.

metal-induced PNA–DNA duplex stabilization origins from non-specific interactions, e.g., intercalation, electrostatic interaction or coordinative bonding with atoms of the DNA backbone. Groove binding is less probable, because it would be mismatch sensitive. Coordination of metal ions to heteroatoms of the third cytosine (from 3′-end) of **13** (Scheme 1) can be excluded, since its replacement with adenine does not affect the melting point of the PNA–DNA duplex. The solid-state structures of Cu^{II}, Ni^{II} and Zn^{II} complexes with *N*,*N*-bis-2-picolyl-gly-gly-OEt have been reported.[15] While the Cu^{II} complex displays square pyramidal structure with N3-in plane coordination and weak axial interaction with amide-O, the Zn^{II} complex is trigonal bipyramidal and the Ni^{II} complex octahedral with two *cis*-oriented free sites. Coordination of DNA phosphodiesters to the vacant in-plane site of the [Cu^{II}(**10**)] complex appears to be sterically hindered by the pyridyl groups. Therefore, the better stabilization of the duplex by Ni^{II} and Zn^{II} could indeed be a consequence of less hindered coordinative interaction of these metal ions with phosphodiester groups of the backbone of the target DNA (Fig. 1). At high NaCl concentration (150 mM) the melting points for **10**:**13** and [Ni^{II}(**10**)] are 52.0 and 56.1 °C, correspondingly. The Ni^{II}-induced stabilization decreases from 7.0 to 4.1 °C, reflecting the relevance of electrostatic interactions for duplex stabilization, which are suppressed at high salt concentration. The nature of the covalent spacer between PNA and $\mathbf{L^3}$ (Gly or Gly-Gly) does not affect the melting temperature in case of metal-free, Zn^{II} and Cu^{II} conjugate, while the gly-gly spacer slightly destabilizes the duplex with the Ni^{II} conjugate (Table 1).

In conclusion, DNA affinity of the conjugates of bis-2-picolylamine with PNA is strongly dependent upon Zn^{II}, Ni^{II} and to a lesser extent Cu^{II} presence.

Supporting Information

MALDI-TOF mass spectra of HPLC purified compounds **5**–**8**, **10**–**12** and of **10**:**13** duplex with and without $NiSO_4$.

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9. General method of coupling of amines to polymer bound PNA. Bromoacetyl bromide (8.7 μL, 100 μM) and DIEA (19 μL, 110 μmol) in DMF (1 mL) are added to the resin-bound PNA having free amino group. The resulting suspension is left shaking for 1 h, then filtered, the resin washed with DMF (2×1 mL), CH_3CN (2×1 mL) and dried under vacuum (10^{-2} mbar). Amine (100 μM) and DIEA (19 μL, 110 μmol) in CH_2Cl_2 (1 mL) are added to the resin and the resulting mixture is left on a shaker for 8 h. Then the resin is filtered, washed with CH_2Cl_2 (2×1 mL), DMF (2×1 mL) and CH_3CN (2×1 mL), and dried under vacuum (10^{-2} mbar).
10. HPLC purification of **5**–**8**, **10**–**12**. HPLC was on EC 250×4.6 mm Nucleosil 300-5 C4 column at 45 °C. Gradients of 0.1% trifuoroacetic acid (TFA) in CH_3CN (solvent B) in 0.1% TFA in water (solvent A) were used: in 5 min from 0 to 2% B, in 13 min to 20% B, in 10 min 20–95% B, 10 min at 95% B.
11. Characterization data for **5**–**8**, **10**–**12**: **5**: Yield 5.4%; HPLC $R_t=24.0$ min; MALDI-TOF MS for $C_{126}H_{167}N_{62}O_{30}$ $[M+H]^+$: calcd 3030.1, found 3029.5; **6**: Yield 5.8%; HPLC $R_t=22.8$ min; MALDI-TOF MS for $C_{128}H_{170}N_{63}O_{31}$ $[M+H]^+$: calcd 3087.2, found 3087.2; **7**: Yield 3.5%; HPLC $R_t=24.0$ min; MALDI-TOF MS for $C_{125}H_{167}N_{62}O_{30}$ $[M+H]^+$: calcd 3018.1, found 3017.1; **8**: Yield 7.6%; HPLC $R_t=21.6$ min; MALDI-TOF MS for $C_{127}H_{170}N_{63}O_{31}$ $[M+H]^+$: calcd 3075.2, found 3073.9; **10**: Yield 6.9%; HPLC $R_t=22.8$ min; MALDI-TOF MS for $C_{130}H_{175}N_{62}O_{30}$ $[M+H]^+$: calcd 3086.2, found 3086.0; **11**: Yield 5.3%; HPLC $R_t=23.3$ min; MALDI-TOF MS for $C_{132}H_{178}N_{63}O_{31}$ $[M+H]^+$: calcd 3143.3, found 3140.6; **12**: Yield 2.5%; HPLC $R_t=18.4$ min; MALDI-TOF MS for $C_{114}H_{159}N_{58}O_{28}$ $[M+H]^+$: calcd 2789.9, found 2787.8.
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