Template-Directed Assembly of Metallosalen – DNA Hairpin Conjugates

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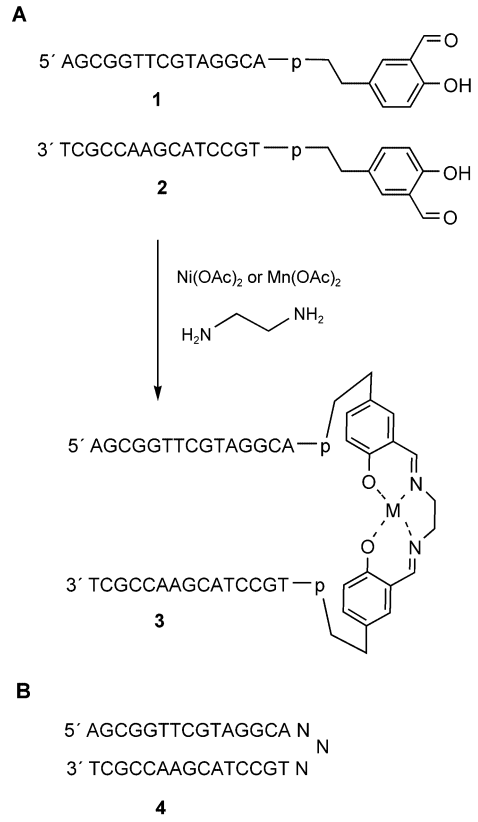
Nucleic acid template-directed synthesis offers a novel method for the encoded assembly of DNA conjugates.[1] DNA template-directed reactions have been developed for chemical ligation of DNA oligonucleotides[2–3] and extended to ligation-based detection systems for point mutations.[4] More generally, templated reactions have successfully directed the synthesis of a remarkable range of chemical structures and reaction classes[1–5] and shown potential for multiplexed synthesis of complex organic molecule – DNA conjugates.[6]

Our laboratory previously demonstrated the template-directed synthesis of a new metal – DNA hybrid, metallosalen – DNA, by diamine cross-linking of salicylaldehyde-modified DNA on an external template.[5] Herein we report the formation and characterization of nickel, manganese, and metal-free salen – DNA conjugates that adopt a hairpin-loop motif (Scheme 1). Metallosalen – DNA hairpin conjugates were designed to determine the three-dimensional structure of metallosalen – DNA hybrids and to evaluate the effect of metallosalen – base pair stacking interactions on DNA duplex stability. Our approach to hairpin metallosalen – DNA conjugates (Scheme 1 A) utilizes two complementary DNA oligonucleotides modified with a salicylaldehyde moiety at either the 3′- or 5′- end (**1** and **2**, respectively). Strands **1** and **2** were aligned by complementary base pairing, which placed the salicylaldehyde groups at the proximal end of a DNA duplex. The aromatic salicylaldehyde moieties were expected to stack with the adjacent base pairs in the duplex,[7] further organizing the salen – DNA reaction. Addition of an appropriate diamine in the presence or absence of a divalent metal ion was expected to produce a hairpin-DNA structure capped by a metallosalen or salen moiety.

Nickel metallosalen – DNA hairpin conjugate (Ni-**3**) formation was assayed by polyacrylamide gel electrophoresis (PAGE) with radiolabeled **1** as a tracer (Figure 1). When strands **1** and **2** were annealed and incubated with 300 μM $Ni(OAc)_2$ and 150 μM ethylenediamine (EN), Ni-**3** was formed in 66% yield after 24 h (lane 1). Extension of the reaction time to 48 h did not enhance the yield of Ni-**3** (lanes 3 and 5). Assembly reactions with strand **2** omitted were performed to assess the rate of unspecific reaction between salicylaldehyde-modified strands (nontemplated, NT).[8]



Scheme 1. *A) Template-directed assembly of metallosalen – DNA hairpins. p = phosphate. B) Sequences of control hairpins with trinucleotide loops (NNN):* ***4a*** *(GAA),* ***4b*** *(TAA), and* ***4c*** *(TTT).*

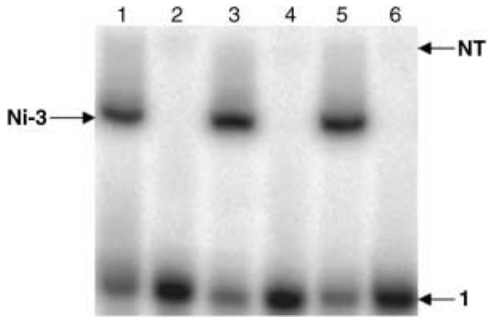


Figure 1. *Time course for Ni-**3** assembly assayed by 20% denaturing PAGE. Lanes 1 – 6: $Ni(OAc)_2$ assembly reactions of **1** and **2** at pH 6.5 with EN. Lane 1: 300 μM $Ni(OAc)_2$, 150 μM EN, 2 μM each **1** and **2** for 24 h at 37 °C. Lane 2: without **2**, 24 h. Lane 3: all, 34 h. Lane 4: without **2**, 34 h. Lane 5: all, 48 h. Lane 6: without **2**, 48 h.*

Notably, NT products (lanes 2, 4, and 6) were detected only after 48 h and at very low levels (1%). Thus, Ni-**3** assembly occurred in a template-directed fashion in 24 h. Similar results were obtained with $Mn(OAc)_2$ in place of $Ni(OAc)_2$. However, as observed in our previous work,[5] manganese metallosalen – DNA hairpin (Mn-**3**) formed more rapidly: 55% yield in 1 h, with NT products becoming visible within 24 h (9%).

Previously, we demonstrated that metallosalen – DNA assembly on an external template required the presence of a metal ion, a diamine, and pH conditions that provided appropriate metal speciation.[5] Interestingly, templated salen – DNA hairpin (**3**)

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formation was observed both in the presence and absence of divalent metal ions. Furthermore, unlike earlier studies, metal-free salen – DNA (EN-**3**) assembly was pH independent, as shown in Figure 2. EN-**3** synthesis yields remained relatively consistent

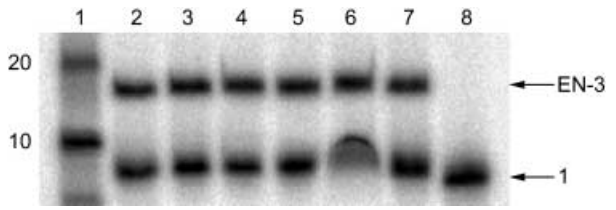

Figure 2. *pH independent assembly of EN-**3** assayed by 20% PAGE. Lane 1: 10 bp marker. Lane 2: pH 6.5, 42%. Lane 3: pH 7.0, 44%. Lane 4: pH 7.5, 47%. Lane 5: pH 8.0, 42%. Lane 6: pH 8.5, 46%. Lane 7: pH 9.0, 36%. Lane 8: radiolabeled **1**.*

(42 – 47%) from pH 6.5 to 8.5 (lanes 2 – 6), but decreased slightly at pH 9.0 (36% yield, lane 7). The absence of a metal-template effect in EN-**3** formation suggests that the salen – DNA assembly proceeds more efficiently in the preorganized hairpin context. Furthermore, EN-**3** may offer a starting point for the synthesis of new metallosalen – DNA conjugates in the presence of diverse metal ions.

The helical properties of the three hairpin salen – DNA conjugates, Ni-**3**, Mn-**3**, and EN-**3**, were investigated by circular dichroism (CD) spectroscopy. For comparison, three hairpin-loop constructs (**4** in Scheme 1 B), in which the metallosalen/salen moiety was replaced with a three-nucleotide loop (**4a**, GAA; **4b**, TAA; **4c**, TTT), also were analyzed by CD. Representative CD spectra for Ni-**3** and **4a** are compared in Figure 3 A. The spectra of each hairpin show a positive band at 280 nm and a negative band at 250 nm, indicative of a B-form helix.[9] All salen – DNA hairpin conjugates gave similar CD spectra (see Supporting Information). Thus, replacement of a DNA hairpin loop with a salen linker does not interfere with the B-form helical nature of the hairpin. One notable feature of Ni-**3** was a minor red shift of the 280 nm band; this may indicate an induced CD signal in the metallosalen-ligand absorption band.[10–11]

The thermal stability of the metallosalen – DNA hairpins was assessed by UV-thermal denaturation studies. UV melting profiles for Ni-**3** and **4a** (Scheme 1 B, X = GAA) are shown in Figure 3 B. Hairpin conjugate Ni-**3** demonstrated a sharp melting transition at $90.5 \pm 0.3\,^{\circ}C$[12] and exhibited greater stability than the control hairpin **4a**, which melted at $T_m = 88.3 \pm 0.3\,^{\circ}C$. The T_m of Mn-**3** ($89.8 \pm 0.4\,^{\circ}C$) was higher than that of the analogous control hairpins **4b** ($86.4 \pm 0.2\,^{\circ}C$) and **4c** ($86.2 \pm 0.2\,^{\circ}C$).[13] The T_m's for metallosalen – DNA hairpins were consistently higher than those for the control hairpins; this suggests that the metallosalen enhances duplex stability through increased stacking interactions, as seen in other capped hairpins.[11, 14]

In summary, we have demonstrated the efficient template-directed synthesis of hairpin metallosalen – DNA conjugates (Ni-**3** and Mn-**3**) and a metal-free salen – DNA (EN-**3**). Template-directed synthesis of hairpin structures, a demonstrated approach for the synthesis of DNA – organic molecular conjugates,[15] has been extended to metal – DNA hybrids. The pH-

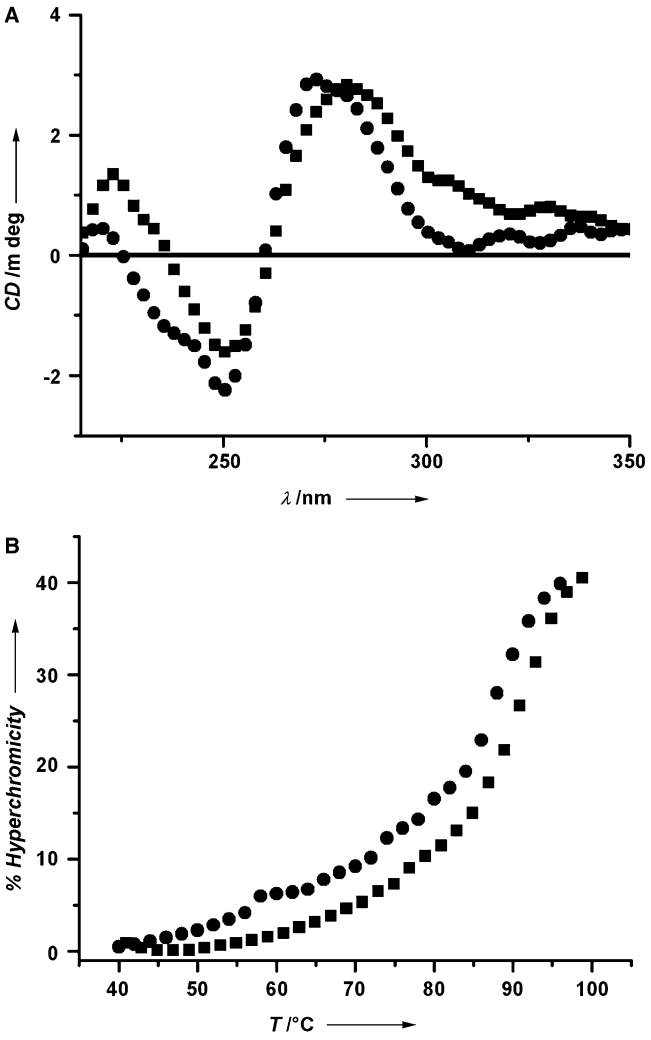

Figure 3. *Biophysical properties of metallosalen – DNA Ni-**3** (squares) and unmodified DNA hairpin **4a** (Scheme 1, X = GAA, circles). A) CD spectra. Conditions: 1 μM hairpin, 10 mM sodium phosphate, 150 mM NaCl, pH 7.0. B) UV-thermal denaturation data was acquired under conditions identical to (A) at 260 nm with a temperature ramp rate of 0.5 °C min^{-1}.*

independentsynthesis of EN-**3** might provide a new route to metallosalen – DNA conjugates with greater metal ion diversity. Hairpin metallosalen – DNA conjugates adopt B-form duplexes that have enhanced thermal stability when compared to DNA hairpins with trinucleotide loops. These new hairpin conjugates offer promise for future studies designed to reveal the structural basis for the enhanced stability of metallosalen – DNA hairpin conjugates.

Experimental Section

Salen – DNA Formation (Ni-3, Mn-3 and EN-3): Buffered solutions of $Ni(OAc)_2$ and EN (final concentrations: 300 μM metal, 150 μM EN, 2 μM each of **1** and **2**) were added to a mixture of **1** and **2** (4 μM each) in MES (10 mM, pH 6.5, 150 mM NaCl). The reaction was incubated for 24 h at 37 °C. Mn-**3** assembly followed a similar procedure. However, the reaction conditions included HEPES buffer (10 mM, pH 8.0, 150 mM NaCl), final reactant concentrations of 400 μM $Mn(OAc)_2$

and 100 μM EN, and 1 h incubation at 37 °C. EN-**3** formation was performed in HEPES buffer (10 mM, pH 7.0, 150 mM NaCl), with a final EN concentration of 400 μM at 37 °C for 1 h. Mn-**3**, Ni-**3**, and EN-**3** provided the expected results by MALDI-TOF MS and base composition analysis (see Supporting Information).

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Keywords: DNA hairpin · metal complex · metallosalen – DNA · nucleic acids · template synthesis

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[12] Concentration-dependent UV thermal denaturation analysis of Ni-**3** gave unchanged T_m values, suggesting that Ni-**3** adopted a hairpin structure (see Supporting Information).

[13] The loops chosen, in increasing stability, were TAA (**4c**), TTT (**4b**), and GAA (**4a**): S. Yoshizawa, G. Kawai, K. Watanabe, K. Miura, I. Hirao, *Biochemistry* **1997**, *36*, 4761. See Supporting Information for melting profiles of all hairpins.

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An Unprecedented Catalytic Motif Revealed in the Model Structure of Amide Hydrolyzing Antibody 312d6

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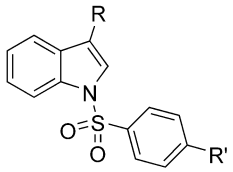
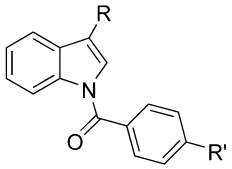
While catalytic antibodies are well established as artificial catalysts with enzyme-like properties for a variety of reactions,[1] few amidase antibodies have been obtained so far.[2] Recently, we have described the preparation and kinetic characterization of amidase antibody 312d6.[3] The antibody was raised against the sulfonamide hapten **2a**, designed to induce catalysis by transition-state mimicry and torsional activation, and has been found to accelerate the hydrolysis of amides **3a**, **4a** by a factor of 10^3 (Scheme 1).





1 R = H
2 R = CHO

3 R = H $k_{cat}/k_0 > 750$ (**3a**)
4 R = CHO $k_{cat}/k_0 = 1120$ (**4a**)

R' = **a**: CH_3; **b**: H; **c**: OCH_3; **d**: Cl; **e**: NO_2

Scheme 1. *Sulfonamide haptens (**1**, **2**) and carboxamide substrates (**3**, **4**) of antibody 312d6.*

Here we report the results of a docking analysis carried out on the homology model of the variable fragment (Fv) region of antibody 312d6. A novel catalytic motif based on an arginine dyad, unprecedented in antibody hydrolases, is identified by this study.

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