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Site-specific oxidative cleavage of DNA by metallosalen–DNA conjugates†

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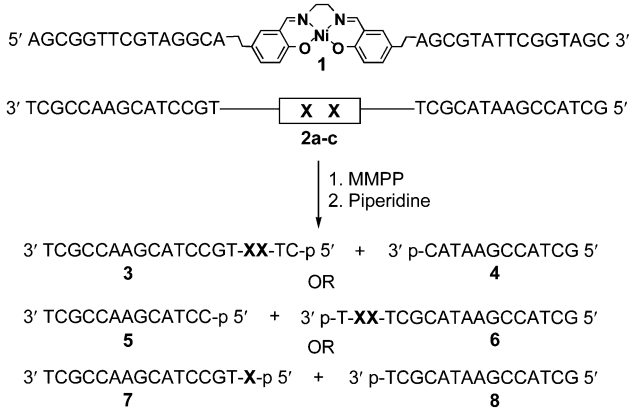
Ni-salen–DNA conjugates, prepared by template-directed synthesis, targeted oxidative adduct formation and strand scission at deoxyguanosine sites in complementary DNA strands of Watson–Crick duplexes.

Redox-active metal complexes may react with double-stranded DNA to produce oxidative damage lesions at sugar and nucleobase sites in DNA nucleotides.[1,2] Metallosalen complexes, well-known for their utility in oxidative synthetic transformations,[3] promote nucleic acid cleavage by oxidative mechanisms.[2,4,5] For example, Mn(III)-salen complexes induce DNA cleavage at A/T-rich regions through minor groove interactions.[4] In contrast, Ni-salens covalently modify guanine nucleotides, which undergo DNA strand scission upon alkaline treatment.[5] Metal–DNA hybrids, in which redox-active metal complexes were conjugated to DNA oligonucleotides, have demonstrated potential as "chemical nucleases" for the targeted oxidative cleavage of nucleic acids.[6]

Our laboratory recently described the assembly of a new metal–DNA hybrid, metallosalen–DNA, using DNA template-directed synthesis.[7,8] Herein, we report metallosalen–DNA's utility for the site-specific cleavage of complementary DNA sequences. Our approach to metallosalen–DNA oxidative cleavage of DNA is illustrated in Scheme 1. Nickel metallosalen–DNA (**1**) is hybridized to a complementary DNA oligonucleotide (**2**) by Watson–Crick base pairing. DNA strand scission is induced by treatment of the duplex (**1**·**2**) with an oxidant, followed by piperidine cleavage at the resulting adduct site. We demonstrate that Ni-salen–DNA **1** produces double stranded DNA adducts that are cleaved by piperidine treatment. The DNA cleavage products (**3–8**) are characterized by gel electrophoresis and mass spectrometry.

Oxidative cleavage of DNA strand **2a** by complementary Ni-salen-**1** was initiated by covalent adduct formation between the strands, which was resolved into DNA cleavage products by alkaline treatment with piperidine (Scheme 1). Ni-salen–DNA **1**[7] was annealed to radiolabeled **2a**, and the oxidation reaction was initiated on the duplex (**1**·**2a**) by addition of magnesium monoperoxyphthalate (MMPP). Denaturing polyacrylamide gel electrophoresis (PAGE) was used to assay the formation of adducts and cleavage products of the reaction (Fig. 1). In the absence of oxidant, no modification of the labeled strand (**2a**) of duplex **1**·**2a** was observed after incubation for 30 min at 37 °C (lane 2). However, when **1**·**2a** was incubated under the same conditions in the presence of 0.9 mM MMPP, DNA products that showed reduced gel mobility (lanes 3–4, 40–50 nt and 60–70 nt bands) were formed. Treatment of the adducted DNA with 600 mM piperidine at 95 °C for 30 min (lane 4) led to the formation of DNA cleavage products.[9] Similar treatment of **2a** without Ni-salen–DNA **1** showed neither adduct nor cleavage product formation (Lane 5). Thus, Ni-salen–DNA **1** facilitated oxidative DNA cleavage by DNA adduct formation and subsequent piperidine treatment.



Scheme 1 DNA cleavage by Ni-salen–DNA **1**. XX = AT (**2a**), GG (**2b**), GC (**2c**). Observed products of DNA strand scission are shown: **3/4** and **5/6** for **2a**, **7/8** for **2b** and **2c**. p = phosphate.

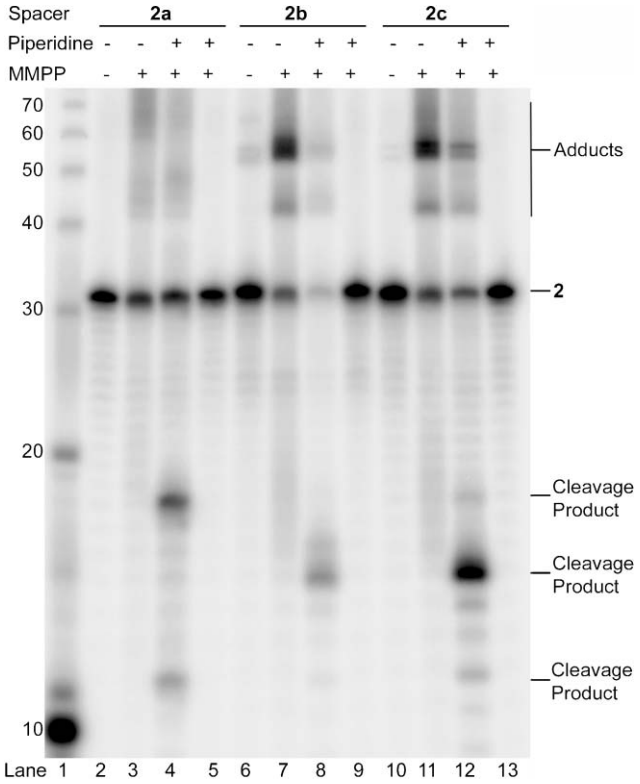


Fig. 1 Gel electrophoresis assay of **2a–c** oxidative cleavage by Ni-salen–DNA **1** with piperidine treatment. 5′-Radiolabeled **2** was hybridized to Ni-salen–DNA **1** and treated at 37 °C for 30 min. Reactions were incubated with 600 mM piperidine for 30 min at 95 °C. Cleavage products were analyzed by 20% denaturing PAGE. Lane 1: 10 bp marker. Lanes 2–13, grouped in four-lane sets: first lane – no oxidant, second – oxidation reaction, third – oxidation and piperidine, fourth – without Ni-salen–DNA **1**. Lanes 2–5, **2a** (AT); lanes 6–9, **2b** (GG); lanes 10–13, **2c** (GC).

† Electronic supplementary information (ESI) available: general experimental protocols, DMS sequencing, PAGE, and MALDI-TOF MS analyses. See http://www.rsc.org/suppdata/cc/b4/b410493e/

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Table 1 MALDI-TOF MS characterization of metallosalen–DNA induced cleavage products of **2**

Strand	Spacer	X	Fragments	$[M]^-$ calcd	$[M]^-$ found
2a	5′-AT-3′	AT	**3**	5804	5800
2a	5′-AT-3′		**4**	3695	3693
2a	5′-AT-3′		**5**	3960	3960
2a	5′-AT-3′	AT	**6**	5539	5532
2b	5′-GC-3′	C	**7**	4883	4882
2b	5′-GC-3′		**8**	4617	4617
2c	5′-GG-3′	G	**7**	4923	4923
2c	5′-GG-3′	G	**8**	4617	4617

Similar adduct formation and DNA cleavage reactions were observed with different target DNA spacers (**2b** and **2c**, lanes 6–9 and 10–13, respectively).

DNA cleavage by metallosalen–DNA paralleled the reactivity of free metallosalen complexes with double-stranded DNA.[5] In particular, a piperidine-labile adduct was formed, which underwent cleavage to form DNA products bearing terminal phosphate groups. The identities of strand **2a** cleavage products (Scheme 1, **3–6**) were verified by two standard methods: comparative PAGE analysis of DNA cleavage fragments from dimethyl sulfate (DMS) dG sequencing reactions[10] and MALDI-TOF MS analysis of the isolated cleavage products. Cleavage products of **2a** from Ni-salen–DNA **1** oxidation migrated at the same mobility as products from DMS sequencing (see ESI). These results suggested that cleavage by **1** occurred selectively at dG residues in **2a** proximal to the metallosalen site in the DNA duplex, and that the 5′-cleavage products of **2a** migrated as phosphate-terminated oligonucleotides. To further characterize the DNA strand scission products, larger scale oxidative cleavage reactions of **1**·**2a** were performed, and the resulting DNA cleavage products were precipitated and desalted.[11] MALDI-TOF MS analysis of the fragments demonstrated their identities as the expected phosphate-terminated oligonucleotides **3–6** (Scheme 1, Table 1).

To assess the target nucleobase preferences of metallosalen–DNA oxidation reactions, cleavage assays were performed on complementary DNA substrates that positioned varied dinucleotides directly across from the Ni-salen in the DNA sequence (Scheme 1, **2a**, AT; **2b**, GG; **2c**, GC). Interestingly, variation of the dinucleotide substrate led to significant alterations in cleavage site selection. As shown in Fig. 1, each substrate (**2a–c**) underwent oxidative cleavage. In all cases, the 5′-cleavage products displayed properties consistent with 3′-phosphorylated DNA fragments.[10] A dG residue positioned directly across from the Ni-salen in **1** (**2b**, GG, lanes 6–9 and **2c**, GC, lanes 10–13) yielded major cleavage products with the same gel mobility (Fig. 1, lanes 8 and 12). In both cases, DMS dG sequencing reactions identified the cleavage site as the 5′-G of the dinucleotide substrate. The cleavage products of **2b** and **2c** were isolated and identified by MALDI-TOF MS as fragments **7** and **8** (Scheme 1, Table 1). In contrast, when the substrate DNA contained a central AT dinucleotide (**2a**), two 5′-cleavage products were observed, which migrated as ~18-nt and ~12-nt fragments.

Remarkably, cleavage of a complementary DNA sequence by metallosalen–DNA maintained specificity for fragmentation at dG residues, as observed in solution with nickel metallosalen complexes.[2d,5] In cases where a guanine base was situated directly opposite the Ni-salen in the substrate DNA (**2b** and **2c**), specific cleavage occurred at that dG site. In contrast, when no guanine base was inserted in the central dinucleotide region (Scheme 1, **2a**, AT) DNA cleavage occurred at dG residues situated in the complementary DNA strand, no more than three residues upstream or downstream of the metallosalen. The optimal yields of cleavage occurred when only one dG residue was across from the Ni-salen in **1** (up to 65% with **2c**), and the yields decreased significantly when the dG residue was further removed from the Ni-salen site (**2a**). These general observations were reproduced in a DNA duplex containing a Ni-salen complex with different flanking DNA sequences (see ESI).

We have demonstrated the efficient site-specific oxidative cleavage of DNA by Ni metallosalen–DNA conjugates. The cleavage reaction required an oxidant for adduct formation between the metallosalen–DNA strand and the target DNA and piperidine for maximal cleavage. Characterization of the DNA cleavage products by gel electrophoresis and MALDI-TOF MS showed that DNA fragmentation produced phosphate-terminated oligonucleotides. Interestingly, Ni-salen–DNA targeted cleavage maintained specificity for dG sites in the complementary strand, even if the nearest dG residue was not directly opposite the metal-complex in the primary sequence. The identity of the DNA adducts formed by oxidative reaction with metallosalen–DNA, the basis of deoxyguanosine specificity, and the DNA cleavage mechanisms are currently under investigation. Metallosalen–DNA may offer a unique approach to DNA cleavage: the template strand, which directs the synthesis of the metal–DNA hybrid, may be selectively cleaved by a subsequent oxidation reaction.

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11 A mixture of **1** and DNA substrate **2** (60 pmol scale) was subjected to strand scission as described. Precipitated DNA fragments were redissolved in water (10 μL), desalted using a Millipore ZipTip®, eluted sequentially with 10%, 25%, and 50% acetonitrile in water, and analyzed by MALDI-TOF MS[7].