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HIGHLY PREFERENTIAL CLEAVAGE OF UNPAIRED GUANINES IN DNA BY A FUNCTIONALIZED SALEN-NICKEL COMPLEX

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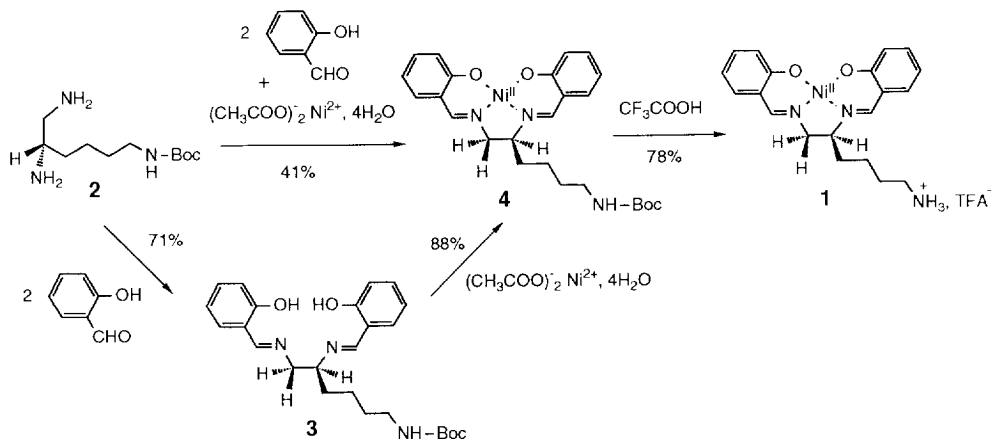
Abstract: In the presence of oxygen donnor compounds, a functionalized salen-nickel complex poorly cuts double-stranded DNA but induces strong cleavages at guanine residues in the single-stranded region of hairpin oligonucleotides. Copyright © 1996 Elsevier Science Ltd

Transition metal complexes that induce damages to nucleic acids provide useful tools in molecular biology to study DNA and RNA structure and function. Phenanthroline-Cu, porphyrin-Mn, EDTA-Fe as well as certain rhodium and ruthenium complexes are among the most frequently used synthetic catalysts to investigate nucleic acids conformation and ligand-DNA interactions.[1]

Derivatives of bis(salicylidene)ethylenediamine, usually referred to as salen, can complex metals and induce DNA cleavage. Different types of DNA damages can occur depending on the chemical reactivity of the complexed metal. Salen-Cu^{II} complexes in the presence of a reducing agent generate oxygen free radicals which attack DNA non specifically;[2] salen-Mn^{III} complexes induce efficient cleavage at AT-rich DNA sequences in the presence of an oxidant;[3] salen-Co^{II} complexes can cleave DNA spontaneously under ambient aerobic conditions[4] and salen-Ni^{II} complexes generally provoke guanine-specific modification of DNA and RNA.[5]

The results reported by others[2-5] and us[6] with salen-metal complexes suggest that the concept of probing nucleic acid structures with salen-based ligands is valid and warrant further development. In this context, we now report the DNA binding and cleaving properties of a functionalized salen-Ni complex **1**.



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Two procedures can be adopted to synthesize the salen-type Schiff base: The nickel complex can be formed directly via reaction of *S*-6-*N*-BOC-1,2,6-triaminohexane **2** with salicylaldehyde in the presence of nickel acetate.[6] Although this procedure proves satisfactory, we found that the alternative route which consists in the formation of the salen prior to complexation with the metal was more convenient. Moreover, the BOC-protected salen derivative **3** provides a versatile reagent for chelating different metals, i.e. Ni, Cu or Mn chosen at will. The BOC-protecting group can be removed with trifluoroacetic acid without destroying the complex.[7] A UV absorption dilution study using the Job method[8] was performed to determine the stability of the salen•Ni complex **4**. This yielded a value of $2 \pm 0.2 \times 10^4$ $dm^3 \times mol^{-1}$ for the complex in aqueous solution. Cyclic voltammetry[9] was used to investigate the redox properties of the salen-Ni complexes incorporating the tetradentate Schiff base. Redox potential (E°) of + 0.635 mV and + 0.645 mV were determined for compounds **4** and **1** respectively.

Figure 1 displays the absorption spectra of compound **1** in the absence and presence of DNA. Significant spectral changes occur attesting that the ligand interacts strongly with DNA. The absorption at 392 nm decreases up to a DNA-phosphate/drug ratio (P/D) of about 10 and then remains constant at higher P/D when the ligand is fully bound to DNA (inset in Fig.1). A marked hypochromism is observed in the 320 nm and 400 nm bands (up to 25% at a DNA/ligand ratio of 20). The interaction of the complex with DNA also causes a bathochromic shift of 16 nm in the visible absorption maximum (from 391 nm to 407 nm, inset in Fig. 1), owing to the perturbation of the complexed chromophore system upon binding to DNA.

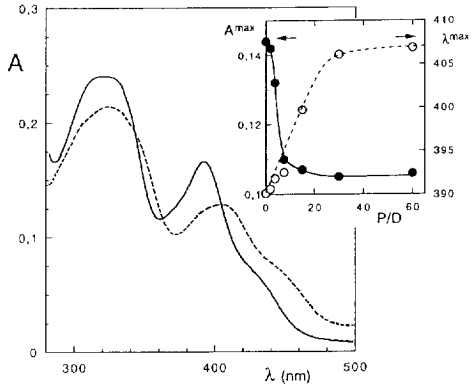

Fig. 1 Absorption spectra of compound **1** (50 μM) in the absence (full line) and presence (dashed line) of calf thymus DNA (1.1 mM). The inset shows the variation of the absorbance A^{max} (●) and the wavelength λ^{max} (O) with increasing DNA-phosphate/compound **1** ratio (P/D).

In the first set of experiments, DNA scission was analyzed using double stranded restriction fragments but practically no cleavage could be detected (data not shown). Then we investigated the cleavage reaction using three complementary 17-mer oligonucleotides 5'-AGTCTAT-XXX-TTAGACT where X=A, C or G. These oligonucleotides form stable hairpin structures (noted hA, hC and hG) with 6 base pairs and an apical loop of 5 bases (Fig. 2). The salen•Ni complex alone does not cleave DNA but the cleavage reaction can be initiated in the presence of oxygen donor compounds such as potassium monoperoxysulfate ($KHSO_5$) and magnesium monoperoxyphtalic acid (MMPP).

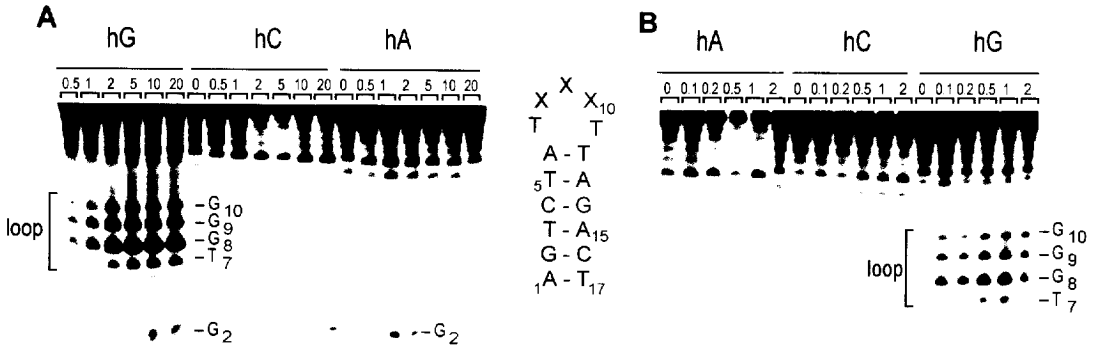

Fig. 2 Cleavage of ^{32}P 5'-end labelled hairpin oligonucleotides (2μM) by compound **1** in the presence of $KHSO_5$ (A) or magnesium monoperoxyphtalate (B). The secondary structure of the 17-mer oligonucleotides hA, hC and hG is indicated. The concentration (μM) of **1** is shown at the top of each gel lane. DNA samples were treated with **1** in the presence of 50 μM $KHSO_5$ or MMPP for 15 min at room temperature and then precipitated with cold ethanol. The DNA pellet was resuspended in 40 μl 1M piperidine, boiled for 12 min at 90 °C and then lyophilized. Cleavage products were resuspended in 5 μl 80% formamide containing 10 mM EDTA and 0.1% tracking dyes. Samples were heated to 90 °C for 4 min and then chilled in an ice-bath just before being loaded on a sequencing gel (15% polyacrylamide, 7 M urea). Major sites of cleavage are marked.

With either $KHSO_5$ (Fig. 2A) or MMPP (Fig. 2B) the cutting occurs exclusively at the GGG triplet of hG. No cleavage can be detected with hC and hA even when using higher salen concentrations and with longer incubation times. The cutting is concentration- and time-dependent. The extent of cutting is already quite large after only 30 sec incubation of DNA with 10 μM compound **1** (Fig. 3). When using a salen-Ni concentration ≥ 5 μM, a faint band can be detected at the paired guanine at position 2 but, as judged from the measured intensities of the bands in each autoradiograms, the extent of cleavage is at least two orders of magnitude lower at G_2 compared to G_8. Therefore, we must conclude that the reaction is specific for accessible guanine residues. The cutting sites where visualized on the autoradiograms after boiling the DNA samples with piperidine. Cleavage can also be detected without alkaline treatment of the oxidized products but in this case the cleavage species are not well resolved on the sequencing gels. By analogy with other related metal complexes, it is plausible to believe that upon activation an active oxidizing species (i.e. an oxosalenato complex) is generated in the viccinity of the DNA target.

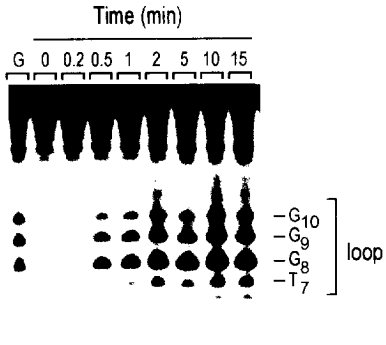

Fig. 3 Time course of the cleavage of the GGG-containing hairpin oligonucleotide (2μM) by compound **1** (10 μM) in the presence of 50 μM $KHSO_5$. Other detail as for Fig. 2.

The above results corroborate the findings of previous studies[5,10] that positively charged salen-Ni complexes can promote efficient cleavage of DNA with a pronounced preference for accessible guanines. The newly introduced butylamino side chain on the salen moiety is now used to tailor peptides and oligonucleotides with the aim of designing sequence-specific chemical nucleases.

References and Notes

1. Sigman, D. S.; Mazumder, A.; Perrin, D. M. *Chem. Rev.* **1993**, *93*, 2295. Barton, J. K. *Science* **1986**, *233*, 727; Chow, C. S.; Barton, J. K. *Methods Enzymol.* **1992**, *212*, 219. Meunier, B. *Chem. Rev.* **1992**, *92*, 1411. Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 746.

2. Sato, K.; Chikira, M.; Fujii, Y.; Komatsu, A. *J. Chem. Soc., Chem. Commun.* **1994**, 625.

3. Gravert, D. J.; Griffin, J. H. *J. Org. Chem.* **1993**, *58*, 820. Gravert, D. J.; Griffin, J. H. *Inorg. Chem.* **1996**, *35*, 4837.

4. Bhattacharya, S.; Mandal, S.S. *J. Chem. Soc., Chem. Commun.* **1995**, 2489.

5. Muller, J. G.; Chen, X.; Dadiz, A. C.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1992**, *114*, 6407; Chen, X.; Woodson, S. A.; Burrows, C. J.; Rokita, S. E. *Biochemistry* **1993**, *32*, 7610; Woodson, S. A.; Muller, J. G.; Burrows, C. J.; Rokita, S. E. *Nucleic Acids Res.* **1993**, *21*, 5524. Cheng, C.-C.; Rokita, S. E.; Burrows, C. J. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 277; Muller, J. G.; Paikoff, S. J.; Rokita, S. E.; Burrows, C. J. *J. Inorg. Biochem.* **1994**, *54*, 199; Burrows, C. J.; Rokita, S. E. *Acc. Chem. Res.* **1994**, *27*, 295.

6. Routier, S.; Bernier, J.-L.; Waring, M. J.; Colson, P.; Houssier, C.; Bailly, C. *J. Org. Chem.* **1996**, *61*, 2326; Routier, S.; Cotelle, N.; Catteau, J.-P.; Bernier, J.-L.; Waring, M. J.; Riou, J.-F.; Bailly, C. *BioOrg. Med. Chem.* **1996**, *4*, 1185.

7. Analytical data for compounds **1**, **2** and **4** are given in ref. 6. Selected spectroscopic data for compound **3**: $[\alpha]^{25}_{D}$ +17 (c 0.04, DMSO); IR (CCl_4) ν 3300, 2940, 1720, 1620 cm^{-1}; MS (FAB$^+$) 4940 $(M+1)^+$; R_f (petroleum ether/Et_2O 50:50) 0.34; ^1H NMR ($CDCl_3$) δ 1.24 (m, 2H), 1.40 (s, 9H), 1.47 (m, 2H), 1.84 (m, 2H), 3.09 (t, J = 6.48, 2H), 3.47 (m, 1H), 3.65 (m, 1H), 3.88 (m, 1H), 4.55 (m, 1H), 6.84 (t, J = 8.5, 2H), 6.90 (d, J = 7.3, 2H), 7.18 (t, J = 7.8, 2H), 7.25 (d, J = 8.0, 2H), 8.27 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 23.37 (CH_2), 28.40 (CH_3), 29.96 (CH_2), 33.69 (CH_2), 40.30 (CH_2), 64.54 (CH_2), 70.10 (Cq), 79.03 (Cq), 116.89 (CH), 118.47 (CH), 131.49 (CH), 132.39 (CH), 156.02 (Cq), 161.00 (Cq), 165.21 (Cq), 166.48 (Cq). Anal. Calcd for $C_{25}H_{33}N_3O_4$: C, 68.30; H, 7.57; N, 9.56. Found: C, 68.64; H, 7.43; N, 9.51.

8. Job, P. *Ann. Chim.* **1927**, 113; Kingery, W. D.; Hume, D. N. *J. Am. Chem. Soc.* **1949**, *71*, 2393.

9. von Veggel, C. J. M.; Bos, M.; Harkema, S.; von de Bonvenkamp, H.; Verboom, W.; Reedijk, J.; Reinhoudt, D. N. *J. Org. Chem.* **1991**, *56*, 225.

10. Muller, J. G.; Zheng, P.; Rokita, S. E.; Burrows, C. J. *J. Am. Chem. Soc.* **1996**, *118*, 2320.

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