Ambient Oxygen Activating Water Soluble Cobalt–Salen Complex for DNA Cleavage

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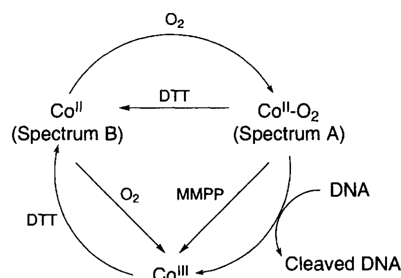
A new water-soluble Co^{II}–salen complex cleaves DNA spontaneously under ambient aerobic conditions; the cleavage is further enhanced by inclusion of 2 mmol dm^{-3} dithiothreitol in the reaction buffer.

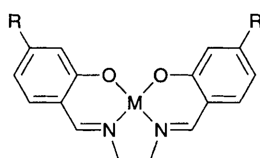
Although many metal complexes capable of promoting DNA strand scission in the presence of cooxidants such as peroxy salts or hydroperoxides have been described,[1] very few of them are known to effect DNA cleavage under *natural aerobic* conditions that prevail within an intact cell. While naturally occurring glycopeptide antibiotics such as bleomycin (BLM)[2] activate ambient molecular oxygen and effect DNA scission, examples of synthetic systems are rare.† The oxygen binding abilities of the metal complex, *N,N′*-ethylene-bis-(salicylidene iminato) (Co–salen), **1a** [M = Co^{II}] are well-known[3] both in the solid state and in solution. However, no attempt so far has been made to exploit this chemistry for DNA cleavage. Herein, we introduce a novel water-soluble, ambient oxygen-activating, bis-cationic Co^{II}–salen complex **1b**‡ that itself cleaves DNA under the physiological conditions.

We first examined DNA binding using the Ni^{II}-complex, **1c**. In the absence of a cooxidant, **1c** does not nick DNA. When examined by UV–VIS absorbance spectroscopy using *Escherichia coli* genomic DNA, decrease (>57% at 258 nm, *ca.* 25% at 381 nm) of the molar extinction coefficients and red-shifts (*ca.* 2–3 nm at 258 nm, *ca.* 10 nm at 381 nm) were observed. Taken together, these findings indicate a strong binding/intercalation[4] by **1c** toward *Escherichia coli* genomic DNA. The binding constant was estimated at $5.3 \pm 0.5 \times 10^5$ dm^3 mol^{-1} using the method described.[5] The complex **1b** when titrated with DNA also gave an indication of initial binding but presumably due to concomitant DNA modification under ambient conditions, reliable estimates of binding constant could not be obtained (see below).

The **1b** induced DNA modifications were examined by performing a primer extension reaction.§ The autoradiogram (Fig. 1) confirms that **1b** itself under natural aerobic conditions modifies plasmid pTZ19R. Lanes 1 and 2 show the modification patterns produced by **1b** on supercoiled and linear plasmid pTZ19R respectively (in a modest G-selective fashion), while lanes 3 and 4 respectively show the controls involving supercoiled and linear DNA that were untreated.

The DNA cleavages by **1b** under ambient conditions (37 °C, pH 7.4) were also examined in the presence of either a cooxidant, magnesium monoperoxyphthalate (MMPP) or a coreductant, dithiothreitol (DTT). We found that in the absence of any external cooxidant or coreductant, [**1b**] > 250 μmol dm^{-3} was required to produce a detectable DNA cleavage at the level of agarose gel. Interestingly, **1b** could not modify DNA at all when MMPP was present (confirmed by examination in sequencing gel). However, in contrast, the DNA scission by **1b** could be profoundly enhanced by performing the cleavage reaction in the presence of DTT. Fig. 2 shows the DNA strand cleavages at various [**1b**] in the presence of 2 mmol dm^{-3} DTT at pH 7.4, 20 mmol dm^{-3} tris HCl and 37 °C (5 min). Note that





1a R = H, M = Co^{II}

b R = $O(CH_2)_3NMe_3^+$, ClO_4^-, M = Co^{II}–O_2

c R = $O(CH_2)_3NMe_3^+$, ClO_4^-, M = Ni^{II}

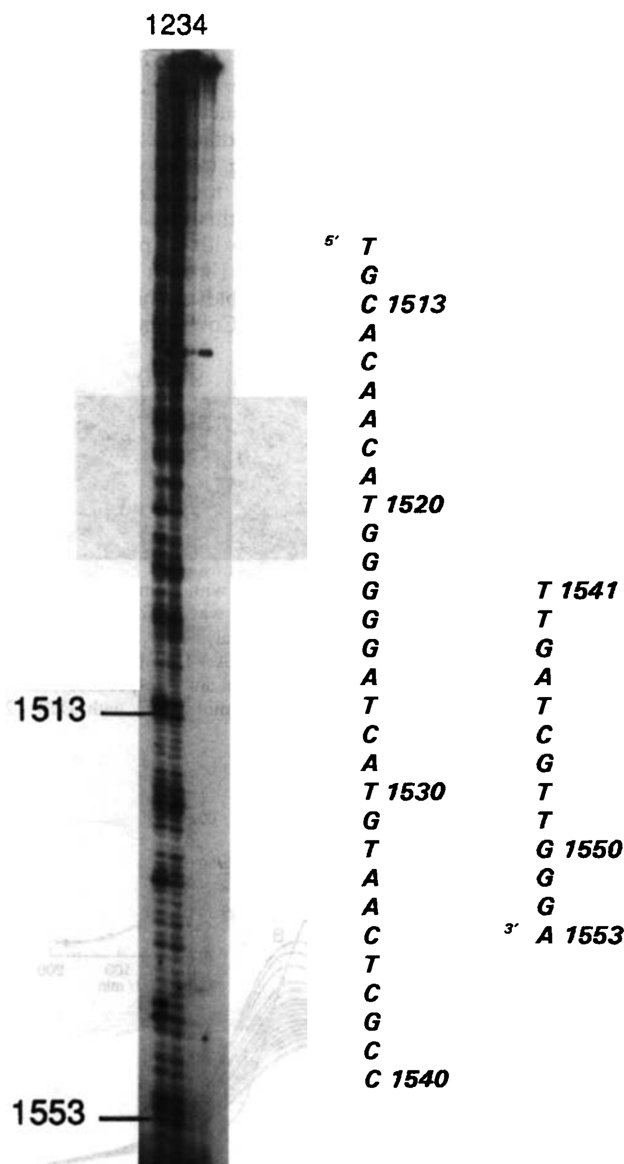


Fig. 1 Autoradiogram of the sequencing gel. Primer extension products of the 5′[^{32}P]-end labelled primer on chemically cleaved fragments were separated in 8% polyacrylamide–bisacrylamide, 50% urea denaturing gel and autoradiogrammed. Lane 1, supercoiled DNA + 50 μmol dm^{-3} **1b**; lane 2, linear DNA + 200 μmol dm^{-3} **1b**. Lane 3, supercoiled plasmid alone and lane 4, linearized (EcoR1) plasmid pTZ19R alone. Cleavage reaction conditions were pH 7.4, 20 mmol dm^{-3} tris.HCl, 5 min, at 37 °C. At the right hand side of the cleavage pattern a portion (1513 to 1553) of the sequences are shown.

even at [**1b**] = 1 μmol dm^{-3}, in the presence of DTT, nicked circular form (F-II) was pro- duced in significant amounts from the supercoiled form (F-I) (lane 7). In the presence of DTT, at [**1b**] = 10 μ mol dm^{-3}, the supercoiled form got almost completely converted to the nicked circular form (F-II) with the generation of some linear form also (F-III) (lane 8). At still higher concentrations of **1b** (50 and 100 μmol dm^{-3}) more pronounced degradation to smaller fragments took place producing smears (lanes 9, 10 respectively). Control experiments verified that neither the reducing agent DTT nor the biscationic salen ligand of **1b** nor Co^{II} ion (alone) induced any detectable DNA cleavage.

What could be the reason for promotion of further DNA cleavage in the presence of DTT? In order to understand this and also to explain the total inhibition of DNA cleavage in the presence of MMPP, we further examined the interaction of **1b** with DTT and MMPP separately by UV–VIS spectroscopy (Fig. 3). In Fig. 3, the spectrum 'A' is due to 0.1 mmol dm^{-3} **1b** in water (λ_{max} = *ca.* 361 nm). Addition of a 10-fold excess of DTT into this solution of **1b** resulted in the formation of a red-shifted absorbance band, 'B' (λ_{max} = *ca.* 378 nm) which gradually decayed to a spectrum 'Q' over a period of time ($t_{1/2}$ = *ca.* 22 min, inset, Fig. 3) in the presence of ambient O_2. In another experiment, MMPP (excess) mediated oxidation of **1b** was studied. The species corresponding to the spectrum 'A' upon oxidation by excess MMPP led to an air-stable end product. The UV-spectral signature of this air-stable product virtually coincided with that of the air-oxidized product of the species generated out of reduction of **1b** with DTT. These results clearly indicate that the air-stable product originates from the formation of the corresponding Co^{III}-complex in either case. Furthermore, the air-stable product could be reverted back to the species corresponding to spectrum 'B' upon addition of excess DTT, which again got converted to the Co^{III} complex under ambient air. So the 'unstable' species corresponding to spectrum 'B' must be due to the free Co^{II}-complex. Therefore, the species corresponding to spectrum 'A', represent neither pure Co^{III}- nor Co^{II}-complex of **1b**. Most likely, spectrum 'A' is due to the oxygen bound Co^{II}-complex. This conclusion is supported from the elemental analysis data of **1b** and also from the fact that it alone can cleave DNA aerobically.

Since the MMPP induced oxidation product [Co^{III}-complex] lacked the O_2 binding ability it also failed to induce DNA nicking. In contrast, however, the DTT reduced product of **1b**, *i.e.* the free Co^{II}-complex, could readily form adducts and thus, cleave DNA as demonstrated in agarose gel.

The above series of experiments thus, not only explain the inability of **1b** toward DNA scission in the presence of MMPP but also provide a clear insight to the observed enhancement of DNA cleavage efficiency when DTT was present. The overall process is summarized in Scheme 1.

In conclusion, the present work represents the first example of the exploitation of oxygen-adducts of Co–salen complexes as potent DNA cleaving agents. These results are particularly significant because they demonstrate that simple water- soluble, Co^{II}–salen–O_2 complexes can induce efficient DNA damage under physiological conditions. No additional reagents, electrochemical activation or photolysis were required for this process. Furthermore, if required, the cleavage efficiencies could be initiated by inclusion of DTT in a buffer.

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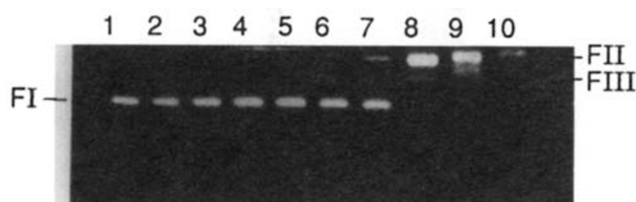

Fig. 2 Cleavage of supercoiled plasmid pTZ19R with **1b** in the presence of DTT. Plasmid pTZ19R DNA (400 ng/reaction) was incubated for 5 min. (37 °C, 20 mmol dm^{-3} tris.HCl, pH 7.4) and analysed by 1% agarose gel-electrophoresis. Lane 1, DNA alone; lane 2, DNA + DTT (2 mmol dm^{-3}); lane 3–6, DNA with [**1b**], 1, 10, 50 and 100 μmol dm^{-3} respectively; lanes 7–10, DNA with [**1b**] 1, 10, 50 and 100 μmol dm^{-3} with DTT (2 mmol dm^{-3}) respectively.

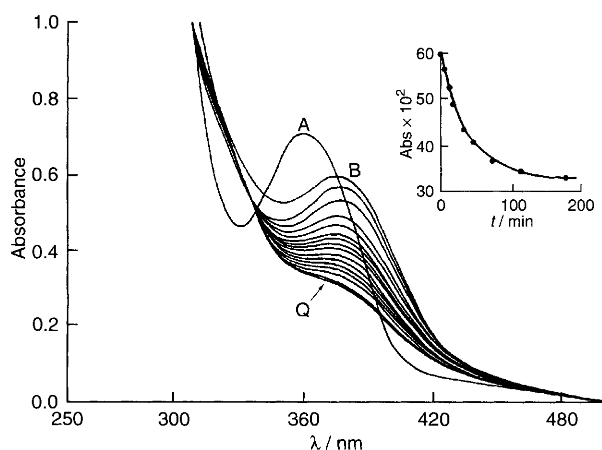

Fig. 3 Reduction of **1b** with DTT and its aerial oxidation: Curve 'A' is due to 1 × 10^{-4} mol dm^{-3} **1b**; Curve 'B' is produced within 1 min of addition of 1 × 110^{-3} mol dm^{-3} DTT into the solution of **1b**; Successively from top (curve 'B') to bottom (curve 'Q') are shown the time-course of this reaction after 3, 8, 13, 18, 23, 28, 33, 43, 53, 63, 73, 93, 113 and 133 min of DDT addition respectively. Inset: exponential decay of DTT reduced product of **1b**.

Footnotes

† See, C-C. Chen, S. E. Rokita and C. J. Burrows, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 277.

‡ Caution! Metal-complexes with perchlorate counterion are potentially explosive. For all the new compounds obtained, the spectroscopic data (^{1}H NMR, UV, IR) and elemental analyses were consistent with their given structures.

§ For details see, S. Sasse-Dwight and J. D. Gralla, *J. Mol. Biol.*, 1988, **202**, 107. Briefly, the reaction products of incubation of plasmid pTZ19R with **1b** for 5 min at 37 °C in 20 mmol dm^{-3} Tris.HCl, pH 7.4 were purified by repeated ethanol precipitations and used as templates for primer extension reaction. A 5′-end[^{32}P]-labelled primer was extended using klenow DNA polymerase till the nick in the phosphodiester backbone produced by cleavage of DNA due to **1b**. The products of this reaction were then analysed on a high resolution denaturing sequencing gel.

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