Cleavage of DNA by nickel complexes

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Abstract

The cleavage of plasmid DNA (pUB110) by several square planar nickel(II) complexes in the presence of either magnesium monoperoxyphthalic acid (MPPA) or iodosylbenzene was investigated. At 25°C and near neutral pH, Ni(salen) (100 μM) or $Ni(CR)^{2+}$ (100 μM) promoted complete conversion of supercoiled plasmid to the nicked circular form in 5 min with iodosylbenzene (0.1 g/ml) as oxidant or in 2.5 h with MPPA (1 mM) as oxidant (salen = bis(salicylaldehyde)ethylenediimine, CR = 2,12-dimethyl-3,7,11,17-tetraazabicyclo[11.3.1]heptadeca-1(17),2,11,13,15-pentaene). No cleavage was observed under similar conditions with $Ni(cyclam)^{2+}$, Ni(dioxocyclam), Ni(TPP) or $Ni(NO_3)_2$ (cyclam = 1,4,8,11-tetraazacyclotetradecane, dioxocyclam = 1,4,8,11-tetraazacyclotetradecane-5,7-dione, TPP = 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine). Possible roles for the nickel complexes in promoting DNA cleavage are discussed.

Introduction

Metal complexes that cleave DNA have been instrumental in the development of sequence-specific DNA cleaving molecules [1]. Complexes such as $Fe(EDTA)^{2-}$ or $Cu(phen)^{2+}$ have been tethered to oligonucleotides [2] or natural products [3] to effect sequence-selective cleavage in the presence of a reducing agent and hydrogen peroxide or molecular oxygen. These reagents generally effect cleavage at multiple nucleotide sites in the vicinity of the sequence recognition agent. This lack of selectivity has been attributed to the production of diffusible oxygen-containing radicals that cleave the DNA. In contrast, it is interesting that a nickel(II) polypeptide complex in conjunction with an oxidant has been shown to effect highly sequence-specific cleavage of DNA [4]. That cleavage occurs at only a small number of sites suggests that cleavage is promoted by a non-diffusible species such as a high-valent nickel complex. Our interest in the development of metal complexes for cleavage of nucleic acids [5] has led us to investigate other nickel complexes which might cleave DNA in the presence of oxidizing agents. Many DNA cleaving agents including iron bleomycin [6] and iron porphyrins [7] have the ability to catalyze olefin epoxidation [8], and we began our search with the nickel complexes that were developed for olefin epoxidation by Burrows and co-workers [9] and Koola and Kochi [10]. A report of the oxidative modification of oligonucleotides by nickel complexes which appeared [11] while this manuscript was in preparation will be discussed in context with our results here.

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Experimental

$Ni(CR)^{2+}$ (**1**) [12], Ni(salen) (**2**) [13], $Ni(cyclam)^{2+}$ (**3**) [9b] and Ni(dioxocyclam) (**4**) [14] were prepared according to literature procedures. Ni(TPP) (**5**) (TPP = 5,10,15,20-tetraphenyl-21*H*,23*H*-porphine was purchased from Aldrich. Either iodosylbenzene prepared according to literature procedures [15] or magnesium monoperoxyphthalic acid (MPPA, Aldrich) were used as oxidants. Solutions were made with Milli-Q purified water. Reagent grade buffers CHES (2-[*N*-cyclohexylamino]ethane sulfonic acid) and potassium hydrogen phosphate were purchased from Fischer Scientific and J. T. Baker Chemicals, respectively. Cacodylic acid was purchased from Schweizerhall, Inc. Solution pH was measured at 25 °C by use of an Orion 510 research digital ion analyzer equipped with temperature compensation probe. ^1H NMR spectra were recorded on a Varian 400 XL spectrometer.

In a typical experiment, plasmid DNA (pUB110, Sigma Chemicals) (0.1 μg/ml) was incubated with 100 μM metal complex with either 0.200 M cacodylic acid, 0.200 M potassium dihydrogen phosphate or 0.100 M CHES buffer at pH = 6.82, 4.95 or 8.65, respectively, at 25 °C. The temperature of the solution was maintained by use of a constant temperature circulator bath. To

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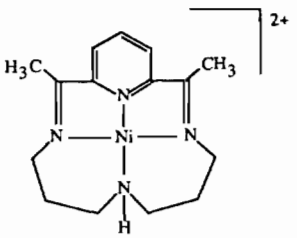
this solution, either 1 mg of iodosylbenzene (0.1 g/ml) or a solution of monoperoxyphthalic acid (1 mM final concentration) was added. Ni(salen) was added as a solution in acetonitrile and final solutions with this complex contained 20% acetonitrile. At the desired time, reactions were quenched by the addition of a solution of bromophenol blue in glycerol and samples were loaded immediately on a 1.4% agarose gel (type II-A medium EEO, Sigma Chemicals). Electrophoresis was conducted on a 10 cm horizontal minigel apparatus (gel thickness of 0.5 cm) at 50 V for 4 h. Electrophoresis buffer was 0.89 M Tris-borate (Tris = tris(hydroxymethyl)aminomethane) at pH 7.75. Gels were stained with 0.5 μg/ml ethidium bromide (Sigma Chemicals).



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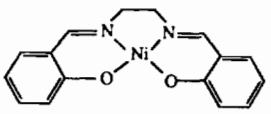

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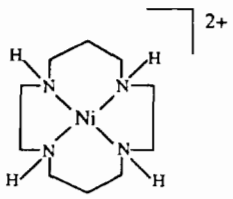

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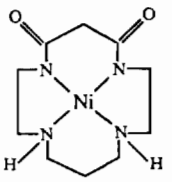

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Results

Conversion of supercoiled plasmid to the nicked open circular form was observed with two nickel complexes, $Ni(CR)^{2+}$ (**1**) and Ni(salen) (**2**) at near neutral pH with MPPA (magnesium monoperoxyphthalic acid) or iodosylbenzene as oxidant at 25 °C (Figs. 1 and 2, respectively). No cleavage was observed under our conditions with $Ni(cyclam)^{2+}$ (**3**), Ni(dioxocyclam) (**4**) or $Ni(NO_3)_2$ with either oxidant or by Ni(TPP) with iodosylbenzene as oxidant (TPP = 5,10,15,20 tetraphenyl-21*H*,23*H*-porphine). No cleavage was observed with oxidants in the absence of nickel complexes (Figs. 1 and 2) nor was cleavage observed with any of the nickel complexes in the absence of oxidant (data not shown). Although the insolubility of iodosylbenzene precluded any direct comparison of the two oxidants at equal concentrations, under our conditions iodosylbenzene promoted more rapid cleavage. Conversion of supercoiled plasmid to the nicked open circular form occurred in minutes with either **1** or **2** in the presence of iodosylbenzene compared to a couple of hours reaction time under similar conditions with MPPA as oxidant. Cleavage reactions with MPPA as oxidant showed a pH dependence with cleavage occurring at similar rates at pH 5.0 and 6.8, but none observed at pH 8.5 (data not shown). NMR experiments suggested that the origin of this pH effect was the instability of MPPA under our reaction conditions; complete decomposition of the peroxyacid to phthalic acid occurred in less than 5 min at pH 8.5.

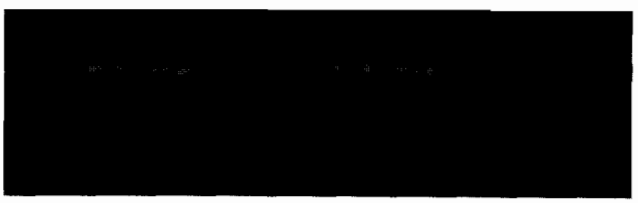

Fig. 1. Effect of 100 μM nickel complex with 1 mM MPPA as oxidant on supercoiled plasmid DNA after incubation at 25 °C for 2.5 h, pH = 6.82. Lanes from left to right: DNA alone, DNA and MPPA, $Ni(NO_3)_2$, Ni(salen), $Ni(cyclam)^{2+}$, Ni(dioxocyclam), $Ni(CR)^{2+}$. The lowermost band is the supercoiled form.

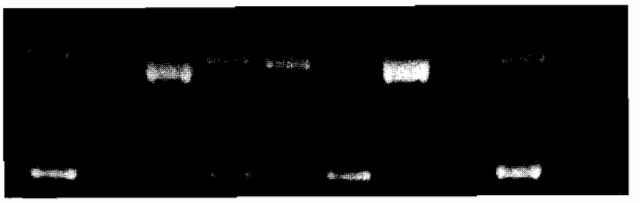

Fig. 2. Effect of 100 μM nickel complex with 0.1 g/ml iodosylbenzene as oxidant on supercoiled plasmid DNA after incubation at 25 °C for 5 min, pH = 7.00. Lanes from left to right: DNA alone, $Ni(NO_3)_2$, Ni(salen), $Ni(cyclam)^{2+}$, Ni(dioxocyclam), Ni(TPP), $Ni(CR)^{2+}$, DNA and iodosylbenzene. The lowermost band is the supercoiled form.

Discussion

The mechanism of metal-promoted oxidative cleavage of DNA has been studied in detail for several metal complexes [6]. The role of the metal may be to generate reactive hydroxy radicals that damage the deoxyribose ring, or alternatively a metal oxygen species may participate directly in oxidation of the deoxyribose ring [16]. Nickel complexes that mediate epoxidation of olefins in the presence of oxygen atom donors presumably do so through the formation of high-valent nickel oxo species [9, 10] (see below). Analysis of events that occur during nickel-mediated DNA cleavage awaits further study of the products which are generated, but nickel oxo complexes may be involved in promoting DNA cleavage as well. Some possible ways that nickel complexes may interact with DNA are suggested below.

Interestingly, cleavage of plasmid DNA by nickel complexes occurred without the addition of base whereas oligonucleotide [11] cleavage by nickel complexes required subsequent treatment with piperidine to observe cleavage. In the case of oligonucleotides, cleavage is mediated by nickel promoted oxidation of guanosine residues; piperidine is then necessary to produce strand cleavage at the damaged guanosine sites. In other systems involving metal promoted oxidations, DNA cleavage may occur through initial attack at the deoxyribose ring and cleavage may be effected without addition of base [6]. One possible explanation for the ready cleavage of supercoiled plasmid by nickel reagents is that the unusual conformation of DNA and its strained form in supercoiled plasmid [17] leads to a different mode of cleavage so that direct strand scission is observed.

High-valent nickel oxo complexes have been invoked as the active catalytic species in nickel complex catalyzed oxidations with oxygen atom donors [9, 10], although with iodosylbenzene as oxidant, an iodosylbenzene complex may be the active oxidizing species [18]. Where high-valent nickel complexes are proposed, correlation between oxidation potential of a nickel complex and its ability to catalyze oxidation has been sought [10]. Similarly, if generation of a high-valent nickel complex is involved here, a correlation between the oxidation potential of a nickel complex and its ability to cleave DNA might be observed. Oxidation of oligodeoxynucleotides by five nickel complexes has been demonstrated to correlate to oxidation potential [11]. In these studies $Ni(CR)^{2+}$ was the most active complex and $Ni(cyclam)^{2+}$ showed slight activity. Although the oxidation potential for the Ni(salen) complex is not reported because of its insolubility in aqueous solution, the higher oxidation potential of $Ni(CR)^{2+}$ $E_{1/2} = 1.03$ V [19] compared to $Ni(cyclam)^{2+}$ (0.67 V) [14], Ni(TPP) (0.56 V) [10], or Ni(dioxocyclam) (0.80 V) [14] suggests that the oxidation potential of the nickel complex may provide a measure of its effectiveness to promote cleavage of DNA. Other considerations that may be important in comparing the ability of nickel complexes to cleave DNA are those governing the binding of nickel complexes to DNA. Binding of a nickel complex to DNA may occur prior to oxidation; $Cu(phen)_2^+$ catalyzed oxidations of nucleic acids are proposed to occur through a copper bound DNA intermediate [20]. Nickel(II) is known to coordinate to both nitrogenous bases and phosphate esters of DNA [21], and coordination of the nickel complexes through an open coordination site to plasmid DNA may play an integral role in the chemistry observed. Recent studies of guanosine specific modification of oligodeoxynucleotides by nickel complexes point to nickel–DNA binding considerations [11]. An overall positive charge on a metal complex might be expected to promote association with DNA. However, we observe that the overall charge on the metal complex is not crucial; both a neutral and a cationic complex promote cleavage. This result stands in contrast to results of a previous report where only nickel complexes bearing a +2 charge effected DNA cleavage [11].

Attachment of nickel complexes that cleave DNA to a sequence-selective recognition agent may create a new sequence-selective cleaving agent for DNA. Studies underway involve the attachment of the CR ligand to recognition agents by taking advantage of the acidic character of the methyl groups on the ligand [22]. However, potential uses of these complexes are limited to *in vitro* applications because of the need to add an external oxidant. More interesting are complexes that catalyze cleavage of nucleic acids without the addition of external reagents as these have more potential as therapeutic agents. Work is underway on this aspect.

Acknowledgements

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