Photolytic Cleavage of DNA by $[Au_3(dmmp)_2]^{3+}$†

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Efficient visible-light induced cleavage of plasmid pBR322 DNA by the long-lived triplet excited state of the polynuclear d^{10} $[Au_3(dmmp)_2]^{3+}$ complex has been found to involve the cleavage of the supercoiled form I to the nicked form II, which may be cleaved further to the linearized form III under carefully controlled conditions.

Studies on the oxidative cleavage of DNA by transition metal complexes are of paramount importance and interest for the design of synthetic restriction enzymes and for the understanding of metal complex–DNA interactions.[1,2] Our recent efforts on the design of novel luminescent polynuclear metal complexes[3,4] with strong oxidizing or reducing properties in the excited state have prompted us to investigate their potential applications in DNA binding and cleavage reactions. In this respect, we have chosen the coordinatively unsaturated polynuclear d^{10} $[Au_3(dmmp)_2]^{3+}$ tricationic complex[4]† and the well characterized plasmid pBR322 DNA as the model system for our studies.

Irradiation of an aqueous solution of $[Au_3(dmmp)_2]^{3+}$ and plasmid pBR322 DNA‡ at $\lambda > 350$ nm ([Au]/[DNA] = 300) in the presence of oxygen for 70 min causes the photocleavage of the supercoiled form of pBR322 to give the linearized pBR322 [lane F, Fig. 1(*a*)]. Lower [Au]/[DNA] ratios of 15 to 150 gave only the nicked form II [lanes B–E, Fig. 1(*a*)]. It is likely that the conversion of form I to linearized form III occurs *via* nicked form II. Increasing the [Au]/[DNA] ratio to >700 under the same photolytic conditions causes the complete degradation of the nicked and linearized forms. However, photolysis of an aqueous solution of the complex and pBR322 with an [Au]/[DNA] ratio of up to 9200 for a shorter period of time (8 min) gave only the nicked form II [lane E, Fig. 1(*b*)]. The effect of the [Au]/[DNA] ratio is depicted in Fig. 1(*b*); the efficiency of conversion to II increases with [Au]/[DNA] ratio (lanes A–E), up to [Au]/[DNA] = 9200. Above this ratio, fading or complete disappearance of the nicked form II occurred, indicative of non-specific multiple cuts [lanes F and G, Fig. 1(*b*)]. Photolytic time-dependent studies for a fixed [Au]/[DNA] ratio also show similar effects; the efficiency of cleavage of supercoiled form I to nicked form II increases with increasing photolysis time, leading to the eventual complete degradation of the plasmid (data not shown).

As seen in the controls, no appreciable cleavage of pBR322 DNA occurs upon irradiation in the absence of $[Au_3(dmmp)_2]^{3+}$ [lane A, Fig. 1(*c*)] or incubation with $[Au_3(dmmp)_2]^{3+}$ in the dark [lane C, Fig. 1(*c*)]. In order to establish the role played by the excited state $[Au_3(dmmp)_2]^{3+}$ complex and oxygen in these DNA photocleavage reactions, we have performed the following experiments.

Irradiation of plasmid pBR322 DNA with $[Au_3(dmmp)_2]^{3+}$ under the same conditions except with the removal of oxygen by bubbling the solution with nitrogen gas resulted in the inhibition of photocleavage (data not shown). However, total inhibition is not observable since complete removal of all traces of oxygen is impossible. It is likely that reactive species such as superoxide radical $(O_2)^{\bullet -}$,[2] singlet oxygen ($^1O_2$),[5] or hydroxyl radical ($^\bullet$OH)[6] are involved in the photocleavage process. Addition of a common $(O_2)^{\bullet -}$ quencher such as superoxide dismutase (SOD) does produce inhibition of photocleavage [lanes D–E, Fig. 1(*d*)].[2] We favour the intermediacy of $(O_2)^{\bullet -}$ at the initial stage of the photocleavage reactions considering the strong reducing properties of the long-lived triplet $[Au_3(dmmp)_2]^{3+*}$ {$E°([Au_3(dmmp)_2]^{4+/3+*}) = -1.6(1)$ V *vs.* SCE; $\tau_0 = 7.0 \pm 0.5$ μs}[4] [eqn. (1)]. The possibility of singlet oxygen involvement at a later stage [eqn. (2)] will be discussed later.

$$[Au_3(dmmp)_2]^{3+*} + O_2 \rightarrow [Au_3(dmmp)_2]^{4+} + (O_2)^{\bullet -} \quad (1)$$

$$[Au_3(dmmp)_2]^{4+} + (O_2)^{\bullet} \rightarrow [Au_3(dmmp)_2]^{3+} + {}^1(O_2) \quad (2)$$

Preliminary transient absorption studies with nanosecond laser flash experiments suggest that the initial reaction of $[Au_3(dmmp)_2]^{3+*}$ with O_2 is electron-transfer in nature, supportive of the mechanism proposed in eqn. (1). To probe the involvement of ($^1O_2$) and/or $^\bullet$OH in the photocleavage

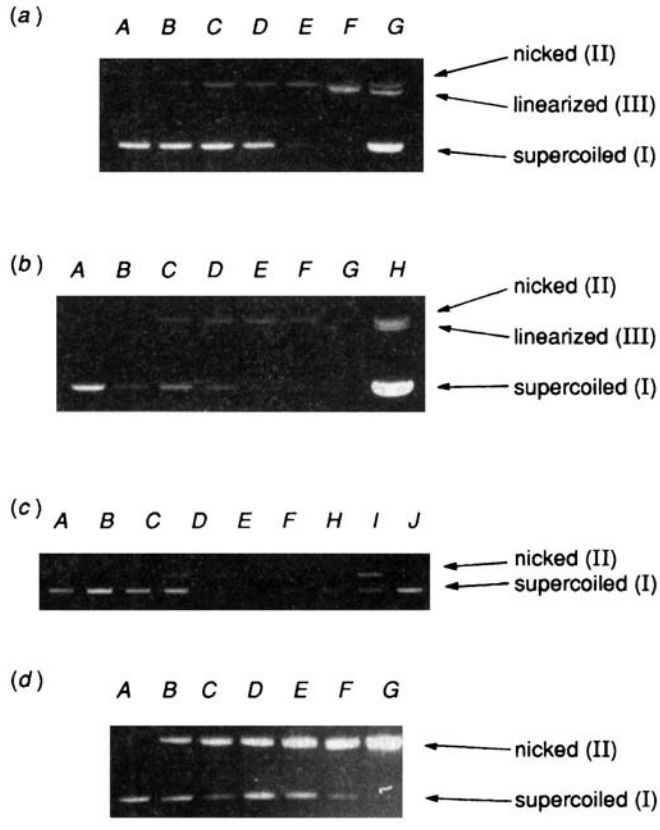

Fig. 1 0.8% Agarose gel showing the results of electrophoresis of pBR322 plasmid DNA (4.6 nmol l^{-1}) photolysed in the presence of $[Au_3(dmmp)_2](ClO_4)_3$. (*a*) 70 min with (*A*) [complex] = 0, (*B*) [complex] = 0.068, (*C*) [complex] = 0.135, (*D*) [complex] = 0.270, (*E*) [complex] = 0.680 and (*F*) [complex] = 1.350 μmol l^{-1}. Lane (G) depicts an authentic sample of forms I–III of pBR322 plasmid DNA. (*b*) 8 min with (*A*) [complex] = 0, (*B*) [complex] = 16.6, (*C*) [complex] = 25.0, (*D*) [complex] = 33.2, (*E*) [complex] = 41.6 (*F*) [complex] = 50.0, and (*G*) [complex] = 83.2 μmol l^{-1}. Lane (H) depicts an authentic sample of forms I–III of pBR322 plasmid DNA. (*c*) 8 min with (*A*) [complex] = 0, (*D*) [complex] = 0.63, (*E*) [complex] = 6.30, (*F*) [complex] = 63.0 μmol l^{-1}. Lanes G–J are under the same condition as (*F*) with the addition of (*G*) [SDS] = 50, (H) [SDS] = 10, (*I*) [NaCl] = 10, (*J*) [NaCl] = 50 mmol l^{-1}. Lanes (*B*) ([complex] = 0 in the dark) and (*C*) ([complex] = 63.0 μmol l^{-1} in the dark) are controls. (*d*) 8 min with (*A*) [complex] = 0, (*B*) [complex] = 6.3, and (*C*) [complex] = 41.6 μmol l^{-1}. Lanes D–F are under the same conditions as in (*C*) with the addition of (*D*) [SOD] = 3.74, (*E*) [SOD] = 0.75 μmol l^{-1}, (*F*) [mannitol] = 50 mmol l^{-1}. Lane (*G*) is under the same conditions as (*B*) except D_2O is used instead of H_2O.

reactions, the following experiments were performed. Photolysis of the metal complex with pBR322 in D_2O did show an enhancement in photocleavage activity [lane G, Fig. 1(*d*)], suggestive of ($^1O_2$) involvement owing to the prolonged lifetime of ($^1O_2$) in D_2O.[5] However, there appeared to be no ·OH involvement in these photocleavage reactions since addition of an excellent ·OH scavenger, mannitol (50 mmol l^{-1}) [lane F, Fig. 1(*d*)] did not show any signs of inhibition.[2] The observation of cleavage to linearized form III *via* an intermediacy of nicked form II at the low metal concentration region [Fig. 1(*a*)] has been ascribed to the controlled generation of low concentrations of active oxidants [($^1O_2$) or $(O_2)^{\cdot -}$], which renders the cleavage more selective. In the high metal concentraton region [Fig. 1(*b*)], initially only the nicked form II is produced which undergoes further non-specific multiple cuts without going through the formation of linearized form III owing to the high local concentration of cleaving species produced upon light irradiation which makes the oxidation process highly non-selective.

The interaction of pBR322 DNA with $[Au_3(dmmp)_2]^{3+}$* has been probed by the following experiments. The efficiency of photocleavage as a function of ionic strength has been investigated. Addition of sodium chloride to the solution produced inhibition of photocleavage activity; its inhibitory ability increased with increasing ionic strength [lanes I–J, Fig. 1(*c*)]. This is suggestive of an electrostatic interaction between the tricationic $[Au_3(dmmp)_2]^{3+}$ and the polyanionic pBR322 DNA; upon light excitation reactive species such as $(O_2)^{\cdot -}$ or ($^1O_2$) are generated close to the target DNA molecule giving rise to a proximity effect. Such an effect would be minimized upon increasing the ionic strength of the solution. This has further been confirmed by a similar inhibition observed with the addition of an anionic surfactant, sodium dodecyl sulfate (SDS) above its critical micelle concentration (cmc) [lanes G–H, Fig. 1(*c*)]. The SDS micelles probably encapsulate the tricationic $[Au_3(dmmp)_2]^{3+}$ complex blocking its interaction with the plasmid pBR322 DNA molecules. A control experiment using the same concentration of NaCl (10 mmol l^{-1}) in place of SDS did not produce any profound inhibition on the photocleavage activity [lane I, Fig. 1(*c*)].

It is interesting to note that the photocleavage of plasmid pBR322 DNA by $[Au_3(dmmp)_2]^{3+}$ can be made to produce either the linearized or the nicked form under carefully controlled conditions. The conversion of supercoiled form I to forms II and III has also been reported with the enediyne compounds.[7] It is likely that at low concentration of the cleaving agent the probability of double-strand scissions is enhanced once the DNA has undergone a single-strand scission, suggestive of a free access of the reactive species to the site of the first cleavage. However, the site of photocleavage is found to be non-specific. Secondary digest of the $[Au_3(dmmp)_2]^{3+}$-linearized pBR322 DNA with the restriction enzyme *Eco* RI shows that the cut is not specific as indicated by the appearance of a smear of bands following enzyme cleavage.

The present work represents the first example of the application of luminescent coordinatively unsaturated polynuclear d^{10} metal clusters as DNA photocleavage agents and demonstrates their capability to induce visible-light photocleavage of plasmid pBR322 DNA.

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Footnotes

† dmmp = bis(dimethylphosphinomethyl)methylphosphine.

‡ $[Au_3(dmmp)_2](ClO_4)_3$ was prepared as previously described.[4] Plasmid pBR322 DNA was extracted from pBR322-transformed *E. coli* DH5a and the supercoiled DNA (form I) purified by density-gradient ultra-centrifugation with ethidium bromide–CsCl to equilibrium.[8] The absorbance ratio at 260 and 280 nm was determined to check for DNA purity.[8] Superoxide dismutase (SOD) from bovine erythrocyte was purchased from Sigma Chemical Co. Solutions were irradiated at room temperature with a 150 W high pressure Hg lamp in Pyrex tubes immersed in a water bath to cut off both UV and IR radiation. Solutions were electrophoresed for 3 h at 40 V on a 0.8% agarose gel in tris acetate buffer, pH 8. The gel was stained with ethidium bromide and photographed under UV light. Secondary digest with *Eco* RI was performed on metal-linearized pBR322 purified from agarose gels.

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