Efficient copper (II)-mediated nuclease activity of *ortho*-quinacridines

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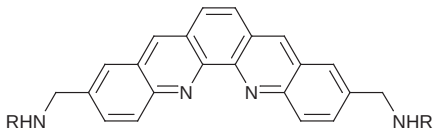
The *ortho*-quinacridine compounds OQ_1 and OQ_2 bind strongly to double stranded DNA and effect efficient cleavage in the presence of Cu^{2+} and in the absence of reducing agent; their activity increases further in the presence of hydrogen peroxide.

Artificial metallonucleases have proven to be efficient tools for the footprinting and sequence-specific targeting of nucleic acids.[1] They are composed of a transition or lanthanide metal ion and of a ligand which plays two major roles: it modulates the reactivity of the metal and interacts with the nucleic acid, delivering the reactive metallic species in the vicinity of the sensitive functionnalities of the biopolymer. Among these compounds, copper(II) complexes were found to cleave DNA, most of them in an oxidative manner.[1–3] They usually require high concentrations of the complex and of an external reducing agent (such as dithiothreitol, DTT) to form *in situ* a reactive copper(I) species, two features which may limit their scope for biological applications at the cellular level. A recent article reports the cleavage of double stranded (ds) plasmid DNA by low concentrations of a tambjamine–copper complex; in this case the ligand is acting as an internal reducing agent.[4]

These considerations prompt us to report on results obtained with water-soluble dibenzo[*b*,*j*]phenanthroline (*i.e.* quinacridine) ligands, fused heteroaromatic pentacycles that display a crescent-shaped structure and were designed to present a large overlap area with pairs or triplets of nucleobases. We recently described their synthesis[5] and their ability to stabilise DNA triple helices through putative intercalation.[6] Among them, the *ortho*-quinacridines (OQ) OQ_1 and OQ_2 possess the *ortho*-phenanthroline (OP) substructure, which forms stable copper complexes. Indeed $Cu(OP)_2^{2+}$ is well known for its ability to cleave nucleic acids oxidatively in the presence of a reducing agent, *via* the formation of Cu(I) and subsequently of copper–oxo species.[1,7] At pH 6, OQ_1 and OQ_2 bear respectively two and



OQ_1, R = $CH_2CH_2CH_3$
OQ_2, R = $(CH_2)_3N(CH_3)_2$

four positive charges located on the ammonium groups of the two side chains.† The ability of OQ to bind to ds DNA was monitored by thermal denaturation experiments and by fluorescence quenching assay[8] (Table 1).

Significant stabilisation of poly(dA.dT)–poly(dA.dT) and efficient displacement of ethidium bromide were observed, indicating that OQ_1 and OQ_2 bind strongly to DNA. OQ_2 was found to be a better DNA ligand than OQ_1 due to its additional positive charges; in addition it seems to have an affinity for DNA comparable to that of the reference compound Hoechst 33258 (stability constant *ca.* 10^7 dm^3 mol^{-1}).[9] Furthermore, the observation of fluorescence contact energy transfer[10] from DNA nucleobases to both dyes gave evidence that intercalation is occurring (data not shown).

Table 1 Interaction of OQ_1 and OQ_2 with Poly(dA.dT)–Poly(dA.dT)

	Ethidium	H33258	OQ_1	OQ_2
ΔT_m[a]/°C	+12	+27	+15	+25.5
C_{50}[b]/μM	—	0.082	0.65	0.081

[a] Variation of the melting temperature of DNA (40 μM in phosphate units) upon addition of the various ligands (6 μM), accuracy ± 1 °C, determined in 30 mM cacodylate buffer (pH 6.0)–10 mM NaCl; the T_m of DNA alone in these conditions was 53.5 °C. [b] Concentration of drug necessary to displace 50% of the DNA-bound ethidium bromide; concentration of DNA 1 μM (in phosphate units) and ethidium 1.26 μM in 30 mM cacodylate buffer (pH 6.0)–10 mM NaCl; the fluorescence emission of ethidium at 595 nm (λ_{exc} = 546 nm) was used to follow this titration.[8]

The ability of OQ to cleave ds plasmid DNA in the presence of Cu^{2+} was then examined (Fig. 1). A slow cleavage was observed (lanes 4–8), with conversion of supercoiled form I to nicked form II at 37 °C over 4 h, in the absence of any reducing agent. In the presence of 100 μM H_2O_2, the nuclease activity of OQ_1–Cu^{2+} and OQ_2–Cu^{2+} was greatly enhanced: Fig. 2 shows a comparison of the cleavage of plasmid DNA (0.4 μg) with low concentrations of OQ or OP (12 μM, [DNA]/[drug] = 10)§ and Cu^{2+} (12 μM), after 5 min incubation at 37 °C, in the absence or presence of 1 mM DTT. In these conditions, all controls were negative including OQ/Cu^{2+} (not shown) and Cu^{2+}/H_2O_2 (lanes 2–3). In the absence of DTT, OQ_1–Cu^{2+} (lane 4) and OQ_2–Cu^{2+} (lane 6) convert effectively form I to forms II (nicked) and III

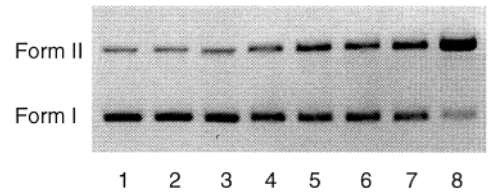


Fig. 1 Cleavage of pUC18 plasmid DNA by OQ_2 in the presence of Cu^{2+}. DNA (0.4 μg) was incubated with OQ_2 in the presence of $CuSO_4$ in 50 mM cacodylate buffer (pH 6.0, total volume 10 μl) at 37 °C and different time. Lane 1, DNA control, 4 h; lane 2, Cu^{2+} 160 μM, 4 h; lane 3, OQ_2 24 μM, 4 h; lanes 4–8, OQ_2 24 μM + Cu^{2+} 160 μM, 15-30-60-120-240 min. OQ_1 exhibited a similar cleavage pattern (data not shown).‡

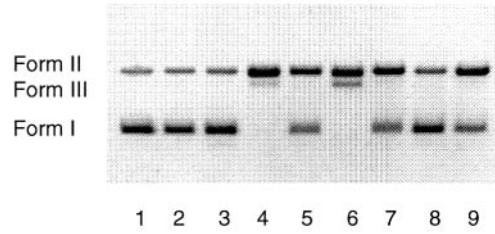


Fig. 2 Cleavage of pUC18 DNA by OQ and OP in the presence of Cu^{2+} and H_2O_2. DNA (0.4 μg) was incubated with the dye in the presence of $CuSO_4$ and H_2O_2 for 5 min at 37 °C in the same buffer as in Fig. 1. Lane 1, DNA control; lane 2, Cu^{2+} 12 μM + H_2O_2 100 μM; lane 3, lane 2 + DTT 1 mM; lane 4, lane 2 + OQ_1 12 μM; lane 5, lane 3 + OQ_1 12 μM; lane 6, lane 2 + OQ_2 12 μM; lane 7, lane 3 + OQ_2 12 μM; lane 8, lane 2 + OP 12 μM; lane 9, lane 3 + OP 12 μM.‡

Table 2 Variation of the absorption maxima of OQ_2 upon addition of Cu^{2+} and H_2O_2[a]

	λ_1	ε_1	λ_2	ε_2	λ_3	ε_3
OQ_2	317	36700	325	34800	—	—
$(OQ_2)Cu^{2+}$	317[b]	29700	328	35400	576	200
$(OQ_2)Cu^{2+}$ + H_2O_2	317[b]	26900	326	29600	577	1400

[a] Wavelength (λ) in nm and molar absorption coefficients (ε) in $dm^3\ mol^{-1}\ cm^{-1}$. Concentration of OQ_2 12 μM in 50 mM cacodylate buffer (pH 6.0); added $CuSO_4$ and H_2O_2 120 μM and 100 μM, respectively. Controls with Cu^{2+} alone (120 μM) and in the presence of H_2O_2 (100 μM) did not show any significant absorption. [b] Present as a shoulder in the major band.

(linear) whereas, as expected, the $(OP)_2Cu^{2+}$ complex (lane 8) does not do so. OQ_2–Cu^{2+} seems more efficient than OQ_1–Cu^{2+} in accordance with the binding affinity of the ligands alone. In presence of DTT, which reduces *in situ* Cu^{2+} to Cu^+, the OP complex becomes active (compare lanes 8 and 9) whereas the activity of the OQ complexes is decreased (lanes 4 to 5 and 6 to 7) though remaining comparable to that of the OP complex. Increasing the time of incubation with OQ_1–Cu^{2+} and OQ_2–Cu^{2+} leads to additional scissions, with increasing percentage of linear form III and appearance of smearing bands.

The efficiency of the DNA cleavage by OQ_2 is dependent on the concentration of copper, with a maximum around 5 equiv. of metal per ligand. A UV titration of OQ_2 by Cu^{2+} indicated the formation of a 1 : 1 complex with a stability constant of 4.7 (in log units). The plateau of the titration is reached at 5 equiv. of Cu^{2+}, in accordance with the copper dependence of the DNA cleavage. The steric hindrance in the vicinity of the two heterocyclic nitrogens prevents a square-planar 1 : 2 Cu^{2+} coordination with OQ_2. Thus a 1 : 1 complex is observed, $(OQ_2)Cu^{2+}$, which is likely the active species responsible for the DNA cleavage. Its relatively weak stability might be ascribed to the effect of the positively charged ammonium linkers of the ligand, which also decreases the basicity of the lone pairs of the heterocyclic nitrogens.[5] By contrast, OP forms a stable square-planar $(OP)_2Cu^{2+}$ complex[11] that is itself unable to cleave DNA. These differences in metal coordination in the $(OQ_2)Cu^{2+}$ and $(OP)_2Cu^{2+}$ complexes might explain the unusual reactivity observed for the former toward DNA. Upon addition of a reducing agent like DTT, the reactive tetrahedral $(OP)_2Cu^+$ is formed *in situ* with subsequent oxidative scission of DNA. The reduced cleavage observed with OQ in these same conditions might be ascribed to the formation of the tetrahedral $(OQ_2)_2Cu^+$ species, which nevertheless retains an activity comparable to that of $(OP)_2Cu^+$ itself.

Table 2 displays the features of the UV spectra of OQ_2 alone and of $(OQ_2)Cu^{2+}$ in the absence and presence of H_2O_2. Upon addition of Cu^{2+} to a solution of OQ_2, a modification of the spectrum is observed with a decrease of the band at 317 nm concomitant to the increase of the band at 325 nm (slightly red-shifted), both bands corresponding to π–π* transitions of the quinacridine moiety; a new band ascribable to d–d transitions of the copper is also emerging at 576 nm. When H_2O_2 is added, a hypochromism of the major band of the ligand (325 nm) is noted along with a hyperchromism of the band at 576 nm, which indicates a strong modification of the metal coordination.

The cleavage of DNA by $(OQ)Cu^{2+}$ complexes and H_2O_2 was inhibited by various radical scavengers: ethanol, thiourea ($HO^\bullet$ scavengers), Tiron ($O_2^{\bullet-}$ scavenger), KI and catalase (H_2O_2 scavengers). These results suggest that the scission mechanism involves the formation of a coordinated peroxide species, with subsequent production of reactive oxygen species that induce oxidative DNA strand scissions. The cleavage in the absence of H_2O_2 (*i.e.* Fig. 1) was not inhibited by reasonable amounts of such scavengers, which does not allow to rule out the intervention of reactive oxygen species.

In conclusion, the $(OQ)Cu^{2+}$ complexes are able to perform an efficient oxidative cleavage of DNA in absence of reducing agent. These new artificial metallonucleases might be interesting lead compounds for the scission of various DNA targets. Further investigations on the reaction mechanism as well as on the structure and sequence selectivity of these reagents are currently underway.

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Notes and references

† All experiments were conducted at pH 6 (cacodylate buffer) where OQ_1 and OQ_2 exist respectively as biprotonated and tetraprotonated species.[5]
‡ All experiments were conducted in the dark in order to avoid photocleavage of DNA by the dyes. Reactions were quenched by adding 1 M KCN and analysed by electrophoresis on 1% agarose gels in 1 M Tris-acetate buffer (80 V, 1 h). The gels were photographed on a UV transilluminator after staining with ethidium bromide.
§ [DNA] in phosphate units.

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