Plasmid DNA cleavage by oxo-bridged vanadium(III) dimers without added co-oxidants or reductants

Tom Otieno,[a] Marcus R. Bond,[a] Ladd M. Mokry,[a] Ronald B. Walter[b] and Carl J. Carrano*[a]

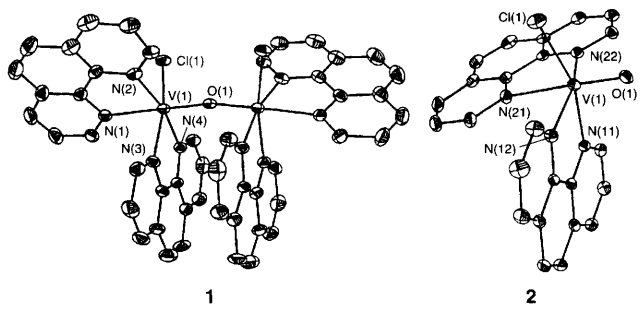
[a] *Department of Chemistry, Southwest Texas State University, San Marcos, TX 78666, USA*
[b] *Department of Biology, Southwest Texas State University, San Marcos, TX 78666, USA*

Plasmid DNA is cleaved by dimeric oxo-bridged vanadium(III) complexes without exogenous oxidants or reductants.

Some naturally occuring antitumour antibiotics such as bleomycin require metal ions as cofactors for DNA strand scission.[1] This has stimulated the development of metal complexes that cleave DNA with the objective of obtaining new pharmaceutical agents, artificial restriction endonucleases, probes for DNA–protein contacts and DNA structure.[2–4] Almost all of these systems require addition of an external agent (*e.g.* light, hydrogen peroxide) to initiate the cleavage and are thus limited to *in vitro* applications. More interesting for potential therapeutic agents would be those that do not require an external oxidant. While the interaction of monometallic complexes with nucleic acids have been widely studied, bimetallic systems have rarely been examined despite the fact that the use of bifunctional reagents is a well developed concept. Bimetallic complexes might be expected to bind strongly to DNA *via* a two-point attachment which in turn could lead to enhanced activity. However, only a few studies on the interaction of dinuclear iron and nickel complexes with DNA have been reported.[5,6] Vanadium(III) oxo-bridged dimers represent a particularly interesting class of complexes to examine for their ability to interact with DNA because the V–O–V unit is quite flexible and easily distorted in response to a number of weak interactions such as π–π stacking and hydrogen bonding.[7] We were attracted to this group of compounds because we felt that such dimers might *adapt* themselves to the DNA groove conformation in such a way as to maximize these secondary interactions. We report here our results with two such compounds of the type $[(VL_2Cl)_2O]^{2+}$ where L = bipyridine or *o*-phenanthroline and show that, not only are they more active DNA cleavage agents than the corresponding monomers, but that they do so in the absence of any externally added agents such as hydrogen peroxide.

The dimeric vanadium(III) complexes $[(VL_2Cl)_2O]^{2+}$, (L = bipy or phen) were prepared in a manner similar to that previously reported, with the corresponding vanadium(IV) monomers obtained by aerial oxidation of the former.[8] The X-ray structures of the two phenanthroline complexes are shown in Fig. 1 and are in general similar to that of the bipyridine analogues.† The slightly smaller bridge angle and smaller intermetallic distance in the phenanthroline dimer presumably results from the increased π-stacking interaction between the phenanthroline rings relative to bipyridine. This highlights the most striking feature of the secondary structure of the dimer which is the proximity of two of the phenanthroline rings coordinated to opposite metals. These rings are nearly parallel and separated by 3.45 Å with a twist angle of about 22.5° between them. This inter-planar spacing is almost perfect for the intercalation of both phenanthroline rings between base pairs in B-DNA, hence a strong interaction between the dimers and DNA could be anticipated. Indeed when supercoiled (form I) pCW8 plasmid DNA was treated with various concentrations of the oxo-bridged vanadium(III) dimers at pH 8.4 and subjected to agarose gel electrophoresis, evidence for strand cleavage was seen. In the case of the bipyridine dimer only single-strand breaks were observable (open circular, form II) and these required millimolar concentrations and long incubation times (hours) to achieve. The phenanthroline dimer on the other hand was far more efficient with both single- and double-strand breaks observable with short incubation times (15 min–2 h) at 10–100 μmol dm^{-3} concentrations. The greater efficiency of the phenanthroline complexes, *vis-a-vis* those of bipyridine, suggests that intercalation is an important factor and we therefore concentrated our further studies on the former. At high concentrations (1–10 mmol dm^{-3}) of the phenanthroline complex, band broadening and slower migration of the plasmid DNA was observed, indicative of binding of the complex to the DNA. Still higher concentrations lead to precipitation of the plasmid DNA as a dark purple solid, due to charge neutralization caused by extensive binding of the cationic phenanthroline dimer.

These dimeric complexes are unusual in that no external reagents such as hydrogen peroxide or ascorbate are required for activation. However, during the course of longer incubations some of the dinuclear vanadium(III) complex was seen to undergo oxidation to the mononuclear vanadium(IV) species. Hence it was possible that either the vanadium(IV) monomer or an intermediate formed during the oxidation of the dimer was the actual active species. Experiments done with the monomer show however that it is in fact less active than the dimer. Furthermore, incubation of the dimer under 'anaerobic' conditions‡ shows no inhibition thus suggesting that oxidation products are also not involved. It should be pointed out that while the anaerobic conditions we employed were sufficient to stop any visible oxidation as evidenced by the lack of a colour change from the deep violet of the dimer to the pale yellow of the monomer, it would be difficult to exclude the presence of a small amount of oxidation. Addition of radical scavengers such as formate or Me_2SO to the reaction mixture, up to a



1 2

Fig. 1 ORTEP view (20% ellipsoids) of the structures of $[\{V(phen)_2Cl\}O]^{2+}$ **1** and $[VO(phen)_2Cl]^{+}$ **2** showing the atom labelling scheme about the metal centres. Selected bond lengths (Å) and angles (°) for **1**: V(1)–O(1) 1.784(1), V(1)–Cl(1) 2.361, V(1)–N(1) 2.194(5), V(1)–N(2) 2.128(6), V(1)–N(3) 2.132(5), V(1)–N(4) 2.136(5), V(1)–O(1)–V(1a) 169.6(3); for **2**: V(1)–O(1) 1.593(3), V(1)–Cl(1) 2.339(1), V(1)–N(11) 2.134(3), V(1)–N(12) 2.120(3), V(1)–N(21) 2.316(4), V(1)–N(22) 2.108(3).

concentration of 1 mol dm^{-3}, only slightly inhibit the cleavage reaction. Likewise 5 μmol dm^{-3} superoxide dismutase had no effect. Catalase was also tested as an inhibitor but unfortunately binds so strongly to the plasmid DNA that it remains in the well during electrophoresis. Taken together these data indicate that the dimer-mediated cleavage reaction in the absence of exogenous hydrogen peroxide does not proceed *via* either diffusible hydroxy radicals or free superoxide. However, when hydrogen peroxide is included in the incubation mixture both monomer and dimer give identical results, that is, extensive cleavage of the plasmid DNA with large amounts of the linear (form III) present indicating double-strand breaks. We attribute this result to the fact that in the presence of hydrogen peroxide the vanadium(III) dimer is rapidly converted to the vanadium(IV) monomer (as determined spectrophotometrically), and vanadyl complexes of various types are known to yield hydroxy radicals in the presence of excess peroxide *via* Fenton type chemistry.[9,10] Thus the mechanisms of cleavage in the presence and absence of hydrogen peroxide appear to be different.

In conclusion we have shown that dimeric μ-oxo vanadium(III) complexes of phenanthroline bind strongly to plasmid DNA, probably by a combination of electrostatic and intercalation effects, and initiate cleavage reactions which do not require the addition of external reagents. These reactions appear not to involve either diffusible hydroxyl radicals or free superoxide ion and are thus different from the Fenton type chemistry seen with vanadium-(III) or -(IV) monomers in the presence of hydrogen peroxide. Further work to elucidate the mechanism and to examine the specificity, if any, of the cleavage reaction on DNA sequence or structure are underway.

We thank Rebecca Obermoeller for excellent technical assistance. The financial support of the Robert A. Welch Foundation (Grant AI-1157 to C. J. C.), the National Institutes of Health through grants CA56728 (R. B. W.) and GM47676-01 (C. J. C.) and The Texas Higher Education Coordinating Board (Grant ARP-029 to R. B. W.) are gratefully acknowledged.

Footnotes

† *X-Ray data for* $[\{V(phen)_2Cl\}_2O]Cl_2 \cdot 8H_2O$ **1**: orthorhombic, space group *Pbcn*, a = 17.950(5), b = 10.574(1), c = 28.817(8) Å, U = 5469.5 $Å^3$, Z = 4, D_c = 1.361 g cm^{-3}, μ(Mo-Kα) = 5.95 cm^{-1}, structure solution and refinement based on 2488 reflections with $2\theta < 45°$ and $|F| > 4\sigma(F)$ converged at R = 0.0666. Note: this structure has apparently appeared as a poster presentation: S. Kitagawa, H. Kiso and M. Munakata, *42nd Symposium on Coordination Chemistry Japan*, 3APO4, Nara, Japan, 1992, but has never appeared in the commonly available literature. Hence we report it here in full. *X-Ray data for* $[VO(phen)_2Cl] \cdot H_2O$ **2**: triclinic, space group $P\bar{1}$, a = 8.440(1), b = 11.454(1), c = 12.347(1) Å, α = 80.249(8)°, β = 79.883(7)°, γ = 73.548(10)°, U = 1117.8(2) $Å^3$, Z = 2, D_c = 1.528 g cm^{-3}, μ(Mo-Kα) = 7.13 cm^{-1}, structure solution and refinement based on 2836 reflections with $2\theta < 45°$ and $|F| > 4\sigma(F)$ converged at R = 0.0478. Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Information for Authors, Issue No. 1.

‡ Anaerobic conditions included incubation in a nitrogen filled glove-bag using degassed buffers.

References

1 J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107.
2 S. J. Lippard, *Acc. Chem. Res.*, 1978, **11**, 211.
3 D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180.
4 A. M. Pyle and J. K. Barton, *Prog. Inorg. Chem.*, 1990, **38**, 413.
5 E. A. Kesicki, M. A. DeRosch, L. H. Freeman, C. L. Walton, D. F. Harvey and W. C. Trogler, *Inorg. Chem.*, 1993, **32**, 5851.
6 L. M. Schaith, R. S. Hanson and L. Que, *Proc. Natl. Acad. Sci. USA*, 1994, **91**, 569.
7 R. S. Czernuszewicz, Q. Yan, M. R. Bond and C. J. Carrano, *Inorg. Chem.*, 1994, **33**, 6116.
8 S. G. Brand, N. Edelstein, C. J. Hawkins, G. Shalimoff, M. R. Snow and E. R. T. Tiekink, *Inorg. Chem.*, 1990, **29**, 434.
9 H. Sakurai, M. Nakai, T. Miki, K. Tsuchiya, J. Takada and R. Matsushita, *Biochem. Biophys. Res. Commun.*, 1992, **189**, 1090.
10 H. Sakurai, H. Tamura and K. Okatani, *Biochem. Biophys. Res. Commun.*, 1995, **206**, 133.

Received, 13th September 1994; Com. 5/060411