Designing molecules for PDT: red light-induced DNA cleavage on disulfide bond activation in a dicopper(II) complex†

Shanta Dhar, Munirathinam Nethaji and Akhil R. Chakravarty*

Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, 560012, India. E-mail: arc@ipc.iisc.ernet.in

The binuclear copper(II) complex $[Cu_2(RSSR)_2]$ (1), where RSSR is a dianionic Schiff base derived from 2-(thioethyl)salicylaldimine having a disulfide bond is prepared, structurally characterized by X-ray crystallography and its photo-induced DNA cleavage activity studied. The Schiff base ligand H_2RSSR is also structurally characterized. The crystal structure of 1 shows the discrete dimeric nature of the complex with each metal showing square-planar geometry with a CuN_2O_2 coordination (Cu···Cu, 5.011(1) Å). The tetradentate Schiff base RSSR acts as a linker of two copper centers. The sulfur atoms in the disulfide unit do not show any apparent interaction with the metal ion. Complex 1, which is cleavage inactive in the dark in the presence of reducing agents, shows significant cleavage of supercoiled pUC19 DNA on exposure to UV light of 312 nm or visible light of different wavelengths under aerobic conditions, in the absence of any additives. DNA cleavage data from control experiments reveal involvement of the disulfide unit as a photosensitizer undergoing photo-induced S–S bond cleavage on exposure to UV light and the resulting species activates molecular oxygen to form singlet oxygen ($^1O_2$) that causes DNA cleavage following a type-II process. Photo-induced DNA cleavage by 1 on red-light exposure using a CW laser of 632.8 nm or a pulsed ruby laser of 694 nm is proposed to involve sulfide radicals in a type-I process and hydroxyl radicals as the reactive species.

Introduction

Natural product antibiotics containing thio-moieties and their synthetic analogues have been used in the DNA cleavage studies.[1–5] Nuclease activity of such sulfur compounds follows thiol-dependent cleavage pathways. The Fenton-type reaction involves activation of molecular oxygen to form hydrogen peroxide which in the presence of a catalytic amount of redox active transition metal ion yields hydroxyl radical as a DNA cleaving agent: $H_2O_2 + M^{n+} \rightarrow HO^{\bullet} + M^{(n+1)+}$. It has been shown that the antitumor antibiotic leinamycin and its analogues act as "chemical nuclease" involving reduction of molecular oxygen to form reactive hydroxyl species.[4,5] We have earlier observed that redox active copper(II) complexes containing ligands having thioalkyl moieties are efficient DNA-cleaving agents on treatment with either a reducing agent or on photo-irradiation.[6] Ternary copper(II) complexes with an equatorial Cu–S bond in a square pyramidal geometry exhibits cleavage of supercoiled (SC) DNA on photo-excitation of the sulfur-to-copper charge transfer band along with the d–d band excitation yielding singlet oxygen.[6b]

Among several types of photosensitizers, those exhibiting red light-induced DNA cleavage activity are of considerable current interest for their potential utility in photodynamic therapy (PDT).[7–9] The basic requirement of PDT is a photosensitizer showing selective retention in the tumor cells, low dark toxicity and photo-activation in the therapeutic window of 650 to 800 nm, an intense light source (generally laser) providing this wavelength, and molecular oxygen. The PDT drug Photofrin® is a porphyrin-based compound that causes DNA damage at 630 nm.[10] The present work stems from our continued interest to develop the chemistry of copper(II) complexes having sulfur containing ligands capable of showing visible light-induced DNA cleavage activity.[6,11,12] The photosensitizing ability of the thio and thione moieties is due to their efficient intersystem crossing leading to the activation of molecular oxygen from its stable triplet to the cytotoxic singlet state.[13,14] Besides, copper is a bio-essential element and copper complexes can be stabilized under different geometrical constraints by suitable ligand design for cellular applications. Copper complexes containing phenanthroline bases are known to show excellent DNA cleavage activity and are used as artificial nucleases in nucleic acid chemistry.[15–20]

We have recently reported a ternary copper(II) complex $[Cu(phen)(met)(MeOH)](ClO_4)$ that has a pendant thiomethyl group of L-methionine (met).[11] This complex containing an α-amino acid shows efficient DNA cleavage on UV light irradiation at 312 nm. However, its DNA cleavage activity is found to be significantly less when exposed to visible light of 532 nm. To investigate the role of the sulfur ligand on the cleavage efficiency, we have now chosen a related Schiff base ligand with a disulfide bond that could be activated on visible light irradiation in a similar way as rhodopsins undergo photo-activation of the S–S bond for its biological function.[21] The formation and cleavage of disulfide bonds are known to be important for the biological activity of several sulfur containing peptides and proteins.[22,23] Compounds containing disulfide linkages have also been used in mapping collagen–protein interactions.[24] In the present work, we have prepared a dicopper(II) complex $[Cu_2(RSSR)_2]$ (1) containing a dianionic tetradentate Schiff base (RSSR) having an exterior disulfide moiety that does not show any apparent bonding interaction with the metal centers. The DNA cleavage reactions are found to proceed *via* different mechanistic pathways on UV or red-light irradiation.

Experimental

Materials

All reagents and chemicals were procured from commercial sources and used without further purifications. Solvents used were purified by standard procedures.[25] Calf thymus (CT) DNA and supercoiled (SC) pUC19 DNA (caesium chloride purified) were purchased from Bangalore Genie (India). Agarose

† Electronic supplementary information (ESI) available: variable temperature magnetic susceptibility data (Table S1), $\chi_M T$ vs. T plot (Fig. S1), metallamacrocyclic ring and the unit cell packing diagrams of 1 (Figs. S2, S3). See http://www.rsc.org/suppdata/dt/b4/b413410a/

(molecular biology grade), 3-mercaptopropionic acid (MPA), dithiothreitol (DTT), ethidium bromide (EB) were from Sigma–Aldrich (USA). The disulfide Schiff base ligand (H$_2$RSSR) was prepared by a literature procedure using cysteamine hydrochloride from Lancaster (UK).[26] The ligand was characterized from the analytical and X-ray structural data. The binary copper(II) complex [CuL$_2$], where HL is 2-(phenylthio)ethylsalicylaldimine, was prepared by a published procedure.[6b]

Physical measurements

The elemental analyses were done using a Heraeus CHN–O Rapid instrument. The IR, electronic and NMR data were obtained from Bruker Equinox 55, Hitachi U-3400 and Bruker AMX 400 spectrometers, respectively. Conductivity measurements were made using Control Dynamics Conductivity Meter. Electrochemical measurements were done at 25 °C on an EG & G PAR model 253 VersaStat potentiostat/galvanostat with electrochemical analysis software 270 for cyclic voltammetric work using a three-electrode setup consisting of glassy carbon working, platinum wire auxiliary and saturated calomel reference electrode. Variable-temperature magnetic susceptibility data in the temperature range 18–300 K were obtained for polycrystalline samples using George Associates Inc. Lewis-coil-force magnetometer system (Berkeley, CA) equipped with a closed-cycle cryostat (Air Products) and a Cahn balance. Hg[Co(NCS)$_4$] was used as a standard. Experimental susceptibility data were corrected for diamagnetic contributions (χ_{dia} = -396×10^{-6} cm^3 mol^{-1} for **1**) and temperature independent paramagnetism ($N_\alpha = 60 \times 10^{-6}$ cm^3 mol^{-1} per copper). The corrected molar magnetic susceptibilities were theoretically fitted by the modified Bleaney–Bowers expression based on the isotropic form of the Heisenberg–Dirac–van Vleck (HDvV) model giving $H = -2J\mathbf{S}_1\mathbf{S}_2$, where $S_1 = S_2 = \frac{1}{2}$.[27,28] The susceptibility equation used was: $\chi_{Cu} = [Ng^2\beta^2/kT][3 + \exp(-2J/kT)]^{-1}(1 - \rho) + (Ng_1^2\beta^2/4kT)\rho + N_\alpha$, where ρ is the fraction of monomeric impurity. The fitting parameters were: $-2J = 5$ cm^{-1}; $g = 2.1$; $g_1 = 2.2$; $\rho = 0.004$.

Synthesis of [Cu$_2$(RSSR)$_2$] (1)

Complex **1** was prepared by reacting CuSO$_4$·5H$_2$O (250 mg, 1.0 mmol) with H$_2$RSSR (360 mg, 1.0 mmol) in CH$_2$Cl$_2$–MeOH (12 cm^3, 5 : 1 v/v) on stirring at 25 °C for 1 h in the dark under nitrogen atmosphere and was isolated in ~70% yield (~300 mg) as a green solid. Found: C, 50.9; H, 4.1; N, 6.9. Calc. for C$_{36}$H$_{36}$Cu$_2$N$_4$O$_4$S$_4$ (**1**): C, 51.2; H, 4.3; N, 6.6%. UV-vis in DMF [λ_{max}/nm (ε/dm^3 mol^{-1} cm^{-1})]: 640 (350), 369 (9420), 306sh (7400), 270 (18700) (sh, shoulder). μ_{eff} (per copper) = 2.0 at 300 and 1.7 μ_B at 18 K. $\Lambda_M = 11$ Ω^{-1} cm^2 mol^{-1} in DMF.

Crystal structure determination

Crystal data for **1**: C$_{36}$H$_{36}$Cu$_2$N$_4$O$_4$S$_4$, $M = 844.01$, triclinic $P\bar{1}$, $a = 8.3080(15)$, $b = 10.6351(10)$, $c = 12.0570(17)$ Å, $a = 102.407(17)$, $\beta = 103.837(12)$, $\gamma = 112.386(19)°$, $U = 899.2(2)$ Å^3, $Z = 1$, $D_c = 1.559$ g cm^{-3}, $T = 293(2)$ K, $\lambda = 0.71073$ Å, $1.9 \leq \theta \leq 25°$, $\mu = 14.61$ cm^{-1}, $F(000) = 434$.

Crystal data for H$_2$RSSR: C$_{18}$H$_{20}$N$_2$O$_2$S$_2$, $M = 360.48$, orthorhombic $P2_12_12_1$, $a = 5.103(3)$, $b = 18.386(12)$, $c = 19.456(13)$ Å, $U = 1825(2)$ Å^3, $Z = 4$, $D_c = 1.312$ g cm^{-3}, $\mu = 3.04$ cm^{-1}.

Data collection and processing. Single crystals of the complex [Cu$_2$(RSSR)$_2$] (**1**) were grown by a diffusion technique by layering petroleum ether on the top of a CH$_2$Cl$_2$ solution of the complex. The ligand H$_2$RSSR was also structurally characterized and crystals were obtained by slow concentration of its methanol solution. Crystal mounting was done on glass fibers using epoxy cement. All geometric and intensity data of **1** were collected using an Enraf-Nonius CAD4 diffractometer equipped with Mo-Kα radiation. Intensity data, collected by ω–2θ scan mode, were corrected for Lorentz and polarization effects, and for absorption.[29a] The intensity data for the Schiff base H$_2$RSSR were obtained from a Bruker SMART APEX CCD diffractometer, having a fine focus 1.75 kW sealed-tube Mo-Kα X-ray source, with increasing ω (width of 0.3° frame^{-1}) at a scan speed of 10 s frame^{-1}. The data were corrected for absorption.[29b]

Structure solution and refinement. The structures were solved and refined with SHELX system of programs.[30] The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were located from the difference Fourier maps and were refined isotropically. The final full-matrix least-squares refinement converged to $R1 = 0.0318$, $wR2 = 0.0889$ for 2780 reflections with $I > 2\sigma(I)$ and 298 parameters [$R1$ (all data) = 0.0384], weighting scheme: $w = 1/[\sigma^2(F_o^2) + (0.0564P)^2 + 0.3742P]$, where $P = [F_o^2 + 2F_c^2]/3$ for **1** and $R1 = 0.0538$ (0.0710 for all data), $wR2 = 0.1094$ ($w = 1/[\sigma^2(F_o^2) + (0.0527P)^2 + 0.0107P]$) for H$_2$RSSR. The goodness-of-fit and the largest difference peak for **1** and H$_2$RSSR are 1.068, 0.432 and 1.176, 0.252 e Å^{-3}, respectively. The perspective views were obtained using the ORTEP program.[31]

CCDC reference numbers 236212 (**1**) and 236213 (H$_2$RSSR).

See http://www.rsc.org/suppdata/dt/b4/b413410a/ for crystallographic data in CIF or other electronic format.

DNA cleavage experiments

Photo-induced cleavage of supercoiled pUC19 DNA by **1** was studied by agarose gel electrophoresis. The reactions were carried out under illuminated conditions using UVITEC transilluminator of 312 nm (96 W, total wattage) or visible monochromatic light of 532 nm (125 W mercury vapor lamp) and 632.8 nm CW He–Ne laser (3 mW, Scientifica-Cook Ltd make, UK). The photolysis was also done using a pulsed ruby laser (Lumonics, 1/6 Hz, 20 ns). Eppendorf and glass vials were used for the UV and visible light experiments, respectively, in a dark room at 25 °C using SC DNA (0.8 µL, 0.5 µg) in 50 mM tris(hydroxymethyl)methane–HCl (Tris-HCl) buffer (pH, 7.2) containing 50 mM NaCl and the complex of varying concentration. After photo-exposure, the sample was incubated for 1 h at 37 °C followed by addition to the loading buffer containing 25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol (3 µL) and the solution was finally loaded on 0.8% agarose gel containing 1.0 µg ml^{-1} ethidium bromide. Electrophoresis was carried out in a dark chamber for 3 h at 40 V in TAE (Tris-acetate-EDTA) buffer. Bands were visualized by UV light and photographed. The extent of cleavage of SC DNA and formation of nicked circular (NC) DNA was measured from the intensities of the bands using the UVITEC Gel Documentation System. Due corrections have been made to the data for the low level of NC form present in the original SC DNA sample and for the low affinity of EB binding to SC compared to the NC form of DNA.[32] Experiments were carried out in the presence of different additives for mechanistic investigations. These reactions were carried out by adding reagents to SC DNA prior to the addition of the complex before photolysis. The concentration of the complex, ligand or the additive corresponded to the quantity of the sample in 2 µL stock solution used. The final concentration was one ninth of the given concentration as the stock solution after adding 0.8 µL SC DNA was diluted with buffer to 18 µL prior to photolysis.

Results and discussion

Synthesis and crystal structure

Complex **1** is prepared in high yield from the reaction of copper sulfate with the disulfide Schiff base H$_2$RSSR in CH$_2$Cl$_2$–MeOH under nitrogen and in the dark (Scheme 1). The complex has been formulated as [Cu$_2$(RSSR)$_2$] from the analytical, spectral
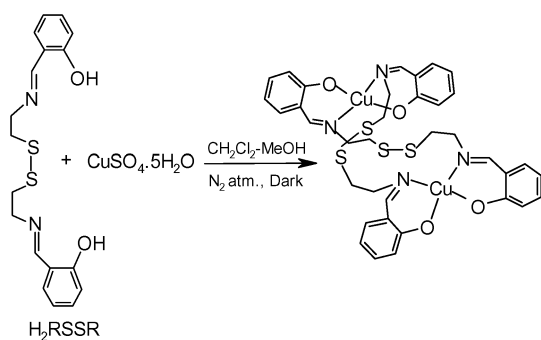
Scheme 1 The reaction pathway for the synthesis of [Cu$_2$(RSSR)$_2$] (**1**)

and magnetic data. It shows a d–d band at 640 nm and a ligand to metal charge transfer band at 369 nm in DMF (Fig. 1). The variable-temperature magnetic susceptibility data in the temperature range 300–18 K show the presence of essentially paramagnetic copper(II) centers ($-2J = 5$ cm^{-1}). Cyclic voltammetric studies in the potential range of 1.5 to -1.5 V do not show any metal-based redox process in DMF/0.1 M TBAP. The complex, however, displays an oxidative response near 0.5 V *vs.* SCE due to the oxidation of the sulfur moiety as is evidenced from the observation of a similar anodic response at 1.16 V in DMF for the ligand H$_2$RSSR.
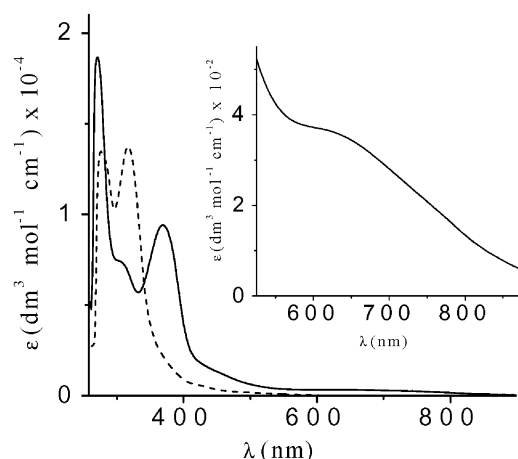

Fig. 1 Electronic spectra of [Cu$_2$(RSSR)$_2$] (**1**) (—) and H$_2$RSSR (---) in DMF with the inset showing the d–d band for **1** (—).

The complex and the ligand H$_2$RSSR are structurally characterized by single-crystal X-ray diffraction. The crystal structure of **1** consists of a dicopper(II) unit having two disulfide Schiff base ligands (Fig. 2(a)). The Schiff base acts as a tetradentate bridging ligand with each metal atom bonded to two donor sites of the ligand. The geometry of the copper atom is essentially square-planar with CuN$_2$O$_2$ coordination and the exterior disulfide moiety is not involved in any apparent metal–ligand interaction. The Cu–N and Cu–O bonds are ~2.0 and 1.9 Å, respectively (Table 1). The S–S distance is 2.024(1) Å. The separation between the two copper centers in the centrosymmetric structure is 5.011(1) Å. The O–Cu–N angles in the basal plane are *ca.* 90°. The complex forms an 18-membered metallamacrocyclic ring.[33] The ligand structure shows a different conformation around the disulfide bond and the phenolic OH group is hydrogen bonded to the imine nitrogen (Fig. 2(b)). The S–S bond length of 2.022(2) Å in H$_2$RSSR is essentially similar to that observed in **1**. The molecular structure of **1** is consistent with the magnetic behavior showing weak magnetic coupling between two copper(II) centers in the absence of any extended π-conjugation in the Schiff base.
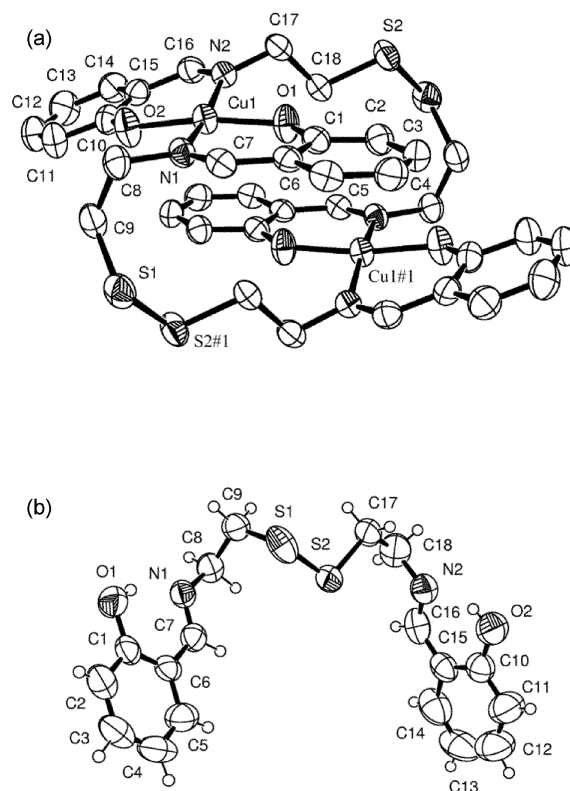

Fig. 2 ORTEP views of [Cu$_2$(RSSR)$_2$] (**1**) (a) and H$_2$RSSR (b) showing the thermal ellipsoids at 50% probability level and the atom numbering scheme.

Table 1 Selected bond distances (Å) and angles (°) for [Cu$_2$(RSSR)$_2$] (**1**) with esds in the parentheses

Cu(1)–O(1)	1.886(2)	O(1)–Cu(1)–O(2)	169.45(10)
Cu(1)–O(2)	1.891(2)	O(1)–Cu(1)–N(1)	92.56(9)
Cu(1)–N(1)	1.998(2)	O(2)–Cu(1)–N(1)	89.02(9)
Cu(1)–N(2)	1.999(2)	O(1)–Cu(1)–N(2)	88.66(9)
S(1)–S(2)	2.024(1)^a	O(2)–Cu(1)–N(2)	91.72(9)
N(1)–C(8)	1.463(3)	N(1)–Cu(1)–N(2)	169.36(9)

^{a} $-x, -y + 1, -z + 1$.

Photo-induced DNA cleavage

The DNA cleavage activity of the complex has been studied using plasmid supercoiled pUC19 DNA (0.5 µg) in a medium of Tris-HCl/NaCl buffer on irradiation with UV light of 312 nm and visible light of different wavelengths such as green light of 532 nm and red light of 632.8 and 694 nm (Figs. 3–5). A 200 µM solution containing 0.33 µg of **1** on 4 h exposure at 532 nm displays essentially complete cleavage of DNA from its SC to the NC form. Control experiments show that addition of sodium azide inhibits the cleavage while the hydroxyl radical scavenger DMSO exhibits minor inhibition. To study the reaction further, we have exposed the sample (90 µM, 0.15 µg) at 312 nm for 20 min and observed complete cleavage of SC DNA (0.5 µg). The complex at this wavelength is cleavage-inactive under argon atmosphere and shows complete inhibition in the presence of azide or histidine, suggesting the possible involvement of singlet oxygen as the reactive species.[7] Addition of DMSO has no apparent inhibiting effect on 312 nm photo-exposure. The involvement of singlet oxygen is again evidenced from significant enhancement of cleavage activity in D$_2$O, in which singlet oxygen has a significantly longer life time than in water.

To investigate the influence of the disulfide moiety in the photo-induced DNA cleavage reaction, we have performed control experiments using a mononuclear Schiff base 2-(phenylthio)ethylsalicylaldimine (HL) copper(II) complex [CuL$_2$] with a CuN$_2$O$_2$ coordination with each ligand containing a pendant
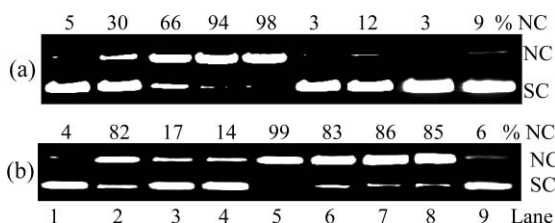
Fig. 3 (a) Gel electrophoresis diagram showing the cleavage of SC pUC19 DNA (0.5 µg) by 1 on UV light (312 nm) irradiation (lanes 1–7) followed by incubation (1 h) in the dark in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing DMF (10%): Lane 1, DNA control (312 nm, 20 min); lane 2, DNA + 1 (90 µM, 10 min); lane 3, DNA + 1 (90 µM, 15 min); lane 4, 1 (90 µM, 20 min); lane 5, DNA + 1 (200 µM, 10 min); lane 6, DNA + H₂RSSR (400 µM, 20 min); lane 7, DNA + [CuL₂] (100 µM, 20 min); lane 8, DNA + 1 (200 µM) + MPA (5 mM), dark; lane 9, DNA + 1 (200 µM) + DTT (5 mM), dark. (b) Gel electrophoresis diagram displaying the cleavage of SC pUC19 DNA (0.5 µg) by 1 (200 µM for all lanes except lane 5 for which the concentration was 100 µM) in the presence of different additives on UV light (312 nm) irradiation for 5 min exposure time in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing DMF (10%). The reactions were carried out under aerobic conditions except for lane no. 9 for which the reaction was done under argon. Lane 1, DNA control; lane 2, DNA + 1; lane 3, DNA + NaN₃ (200 µM) + 1; lane 4, DNA + L-histidine (200 µM) + 1; lane 5, DNA + D₂O (14 µL) + 1 (100 µM); lane 6, DNA + DMSO (4 µL) + 1; lane 7, DNA + mannitol (200 µM) + 1; lane 8, DNA + sodium formate (200 µM) + 1; lane 9, DNA + 1 (under argon atmosphere).
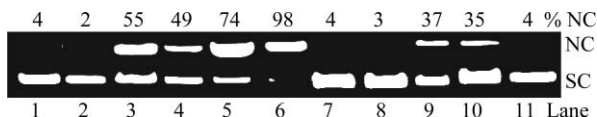

Fig. 4 Gel electrophoresis diagram showing the cleavage of SC pUC19 DNA by 1 using monochromatic radiation of 532 nm at different complex concentrations and exposure times. The reactions were carried out under aerobic conditions except for lane no. 11. Lane 1, DNA control (4 h); lane 2, DNA + 1 (200 µM, in dark, 4 h); lane 3, DNA + 1 (100 µM, 4 h); lane 4, DNA + 1 (200 µM, 1 h); lane 5, DNA + 1 (200 µM, 3 h); lane 6, DNA + 1 (200 µM, 4 h); lane 7, DNA + NaN₃ (200 µM) + 1 (200 µM, 4 h); lane 8, DNA + L-histidine (200 µM) + 1 (200 µM, 4 h); lane 9, DNA + DMSO (4 µL) + 1 (200 µM, 4 h); lane 10, DNA + mannitol (200 µM) + 1 (200 µM, 4 h); lane 11, DNA + 1 (200 µM, 4 h) (under argon).
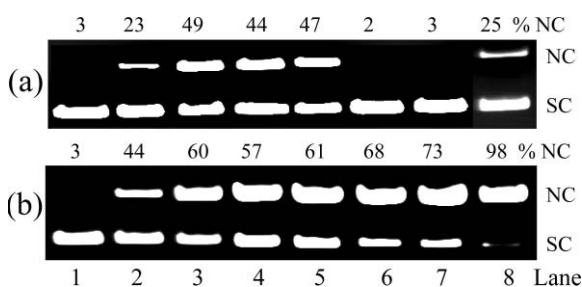

Fig. 5 (a) Red light-induced cleavage of SC pUC19 DNA by 1 (300 µM) in 50 mM Tris-HCl/NaCl buffer (pH, 7.2) containing DMF (10%) using a 632.8 nm CW laser (3 mW) at different exposure times. The reactions were carried out under aerobic conditions except for lane no. 8 for which the reaction was done under argon. Lane 1, DNA control (3 h); lane 2, DNA + 1 (1 h); lane 3, DNA + 1 (3 h); lane 4, DNA + NaN₃ (300 µM) + 1 (3 h); lane 5, DNA + L-histidine (300 µM) + 1 (3 h); lane 6, DNA + DMSO (4 µL) + 1 (3 h); lane 7, DNA + mannitol (300 µM) + 1 (3 h); lane 8, DNA + 1 (3 h) (under argon). (b) Gel diagram showing the cleavage of SC DNA (0.5 µg) by 1 using a pulsed ruby laser (694 nm, 40 mJ pulse⁻¹ peak power): lane 1, DNA control (4 h); lane 2, DNA + 1 (300 µM, 1 h); lane 3, DNA + 1 (300 µM, 3 h); lane 4, DNA + 1 (400 µM, 30 min); lane 5, DNA + 1 (400 µM, 1 h); lane 6, DNA + 1 (400 µM, 2 h); lane 7, DNA + 1 (400 µM, 3 h); lane 8, DNA + 1 (400 µM, 4 h).

SPh moiety.[6b] This complex does not show any significant DNA cleavage on photo-irradiation at 312 nm (Fig. 3(a), lane 7). The ligand H₂RSSR alone is cleavage inactive. Our earlier work has shown that a dinuclear copper(II) complex [{(phen)Cu}₂(µ-

OH)₂](ClO₄)₂ with a CuN₂O₂ coordination but lacking any disulfide moiety is cleavage inactive on UV (312 nm) and red-light (632.8 nm) irradiation.[34] Complex 1 is also cleavage inactive in the dark in the presence of reducing agents such as MPA or DTT (Fig. 3(a), lanes 8, 9). The results indicate the importance of copper, the disulfide moiety in the complex and light in DNA cleavage activity. Photo-exposure at 312 nm is believed to generate an excited triplet state involving charge transfer bands near 300 and 369 nm in conjunction with the disulfide moiety of 1 leading to the formation of ¹O₂ through energy transfer in a type-II process (Scheme 2).[18]
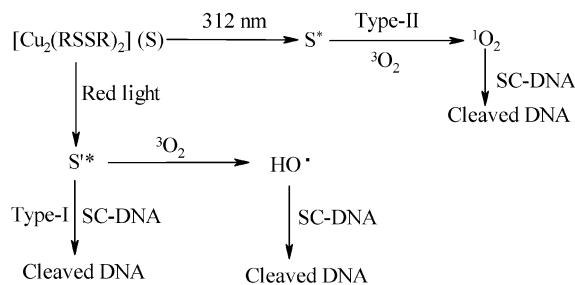
Scheme 2 Mechanistic pathways proposed for the photo-induced cleavage of SC-DNA by [Cu₂(RSSR)₂] (1)

We are particularly interested to explore the photonuclease activity of 1 on red-light exposure considering its utility in designing molecules for PDT. When exposed to a low-power red-light CW laser of 632.8 nm, 300 µM (0.5 µg) complex on 3 h exposure shows ∼50% cleavage of SC DNA. Control experiments reveal that singlet oxygen quencher azide or histidine addition has no effect on the cleavage activity. Addition of the hydroxyl radical scavenger DMSO, however, completely inhibits the cleavage reaction. The photocleavage reactions at UV and red light seem to follow different reaction pathways. While the UV exposure involves the CT bands and disulfide bond, the red-light exposure seems to cause d–d band excitation at 640 nm and disulfide bond cleavage.[35] Based on the control experiments data, we propose that the red-light exposure leads to sulfide radicals which can cleave SC DNA as a major pathway in a type-I process, or might generate hydroxyl radicals (Scheme 2).[18,36] Tapley et al. have shown that sulfide radicals in the presence of molecular oxygen form reactive hydroxyl radicals.[37] Control experiments under argon on 632.8 nm exposure show ∼25% cleavage of SC DNA to its NC form (Fig. 5(a), lane 8). This is significant considering ∼50% cleavage observed under aerobic conditions (Fig. 5(a); lane 3). The results indicate the possibility of both sulfide radical type-I and hydroxyl radical pathways to be operative at this wavelength. We have studied the photocleavage activity of 1 at 694 nm using a ruby laser (40 mJ pulse⁻¹). An essentially complete cleavage of SC DNA is observed with a higher complex concentration and longer exposure time (Fig. 5(b), lane 8).

Conclusions

In summary, we present the first report of a metal-promoted efficient DNA cleavage activity on disulfide photo-activation using a dicopper(II) complex at ∼700 nm. Such a long wavelength is ideally suited for PDT applications considering the greater transparency of human tissue at this wavelength compared to 630 nm used for Photofrin-II®. Our observation of red light-induced DNA cleavage by a copper(II) complex having a disulfide moiety is novel and this work is likely to presage further design and development of analogous metal-based non-porphyrinic photosensitizers using biologically important peptides or related ligands having disulfide linkages to achieve efficient DNA cleavage at lower complex concentrations and for cellular applications in PDT. Observation of DNA cleavage on red-light exposure under anaerobic conditions is significant

considering the low concentration of oxygen in malignant tumor cells.

Acknowledgements

References

1 (a) M. Hara, I. Takahashi, M. Yoshida, K. Asano, I. Kawamoto, M. Morimoto and H. Nakano, *J. Antibiot.*, 1989, **42**, 333; (b) M. Hara, Y. Saitoh and H. Nakano, *Biochemistry*, 1990, **29**, 5676; (c) A. Asai, M. Hara, S. Kakita, Y. Kanda, M. Yoshida, H. Saito and Y. Saitoh, *J. Am. Chem. Soc.*, 1996, **118**, 6802.

2 M. Chatterjee, K. D. Cramer and C. A. Townsend, *J. Am. Chem. Soc.*, 1993, **115**, 3374.

3 (a) N. Tamura Tasaka, M. Matsushita, R. Hayashi, K. Okonogi and K. Itoh, *Chem. Pharm. Bull.*, 1993, **41**, 1043; (b) Y. Kohama, K. Iida, T. Semba, T. Mimura, A. Inada, K. Tanaka and T. Nakanishi, *Chem. Pharm. Bull.*, 1992, **40**, 2210; (c) B. S. Davidson, T. F. Molinski, L. R. Barrows and C. M. Ireland, *J. Am. Chem. Soc.*, 1991, **113**, 4709.

4 S. J. Behroozi, W. Kim, J. Dannaldson and K. S. Gates, *Biochemistry*, 1996, **35**, 1768.

5 K. Mitra, W. Kim, J. S. Daniels and K. S. Gates, *J. Am. Chem. Soc.*, 1997, **119**, 11691.

6 (a) S. Dhar and A. R. Chakravarty, *Inorg. Chem.*, 2003, **42**, 2483; (b) S. Dhar, D. Senapati, P. K. Das, P. Chattopadhyay, M. Nethaji and A. R. Chakravarty, *J. Am. Chem. Soc.*, 2003, **125**, 12118.

7 E. D. Sternberg, D. Dolphin and C. Brückner, *Tetrahedron*, 1998, **54**, 4151.

8 J. L. Sessler, G. Hemmi, T. D. Mody, T. Murai, A. Burrell and S. W. Young, *Acc. Chem. Res.*, 1994, **27**, 43.

9 M. C. De Rosa and R. J. Crutchley, *Coord. Chem. Rev.*, 2002, **233–234**, 351.

10 (a) R. Ackroyd, C. Kelty, N. Brown and M. Reed, *Photochem. Photobiol.*, 2001, **74**, 656; (b) B. W. Henderson and T. J. Dougherty, *Photochem. Photobiol.*, 1992, **55**, 145; (c) B. W. Henderson, T. M. Busch, L. A. Vaughan, N. P. Frawley, D. Babich, T. A. Sosa, J. D. Zollo, A. S. Dee, M. T. Cooper, D. A. Bellnier, W. R. Greco and A. R. Oseroff, *Cancer Res.*, 2000, **60**, 525.

11 A. K. Patra, S. Dhar, M. Nethaji and A. R. Chakravarty, *Chem. Commun.*, 2003, 1562.

12 (a) P. A. N. Reddy, B. K. Santra, M. Nethaji and A. R. Chakravarty, *J. Inorg. Biochem.*, 2003, **98**, 377; (b) B. K. Santra, P. A. N. Reddy, G. Neelakanta, S. Mahadevan, M. Nethaji and A. R. Chakravarty, *J. Inorg. Biochem.*, 2002, **89**, 191; (c) A. M. Thomas, M. Nethaji, S. Mahadevan and A. R. Chakravarty, *J. Inorg. Biochem.*, 2003, **94**, 171.

13 X. Qian, T.-B. Huang, D.-Z. Wei, D.-H Zhu, M.-C. Fan and W. Yao, *J. Chem. Soc., Perkin Trans. 2*, 2000, 715.

14 (a) A. Jakobs and J. Piette, *J. Photochem. Photobiol. B*, 1994, **22**, 9; (b) A. Jakobs and J. Piette, *J. Med. Chem.*, 1995, **38**, 869; (c) A. Seret, J. Piette, A. Jakobs and A. Van de Vorst, *Photochem. Photobiol.*, 1992, **56**, 409.

15 (a) D. S. Sigman, T. W. Bruice, A. Mazumder and C. L. Sutton, *Acc. Chem. Res.*, 1993, **26**, 98; (b) D. S. Sigman, A. Mazumder and D. M. Perrin, *Chem. Rev.*, 1993, **93**, 2295; (c) D. S. Sigman, *Acc. Chem. Res.*, 1986, **19**, 180.

16 (a) D. S. Sigman, D. R. Graham, V. D. Aurora and A. M. Stern, *J. Biol. Chem.*, 1979, **254**, 12269; (b) O. Zelenko, J. Gallagher and D. S. Sigman, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 2776.

17 (a) G. Pratviel, J. Bernadou and B. Meunier, *Adv. Inorg. Chem.*, 1998, **45**, 251; (b) B. Meunier, *Chem. Rev.*, 1992, **92**, 1411; (c) M. Pitié, C. Boldron, H. Gornitzka, C. Hemmert, B. Donnadieu and B. Meunier, *Eur. J. Inorg. Chem.*, 2003, 528.

18 W. K. Pogozelski and T. D. Tullius, *Chem. Rev.*, 1998, **98**, 1089.

19 (a) S. A. Ross, M. Pitié and B. Meunier, *Eur. J. Inorg. Chem.*, 1999, **557**; (b) C. Boldron, S. A. Ross, M. Pitie and B. Meunier, *Bioconjugate Chem.*, 2002, **13**, 1013; (c) S. Routier, V. Joanny, A. Zaparucha, H. Vezin, J.-P. Catteau, J.-L. Bernier and C. J. Bailly, *J. Chem. Soc., Perkin Trans. 2*, 1998, 863.

20 S. Dhar, D. Senapati, P. A. N. Reddy, P. K. Das and A. R. Chakravarty, *Chem. Commun.*, 2003, 2452.

21 J. Saranak and K. W. Foster, *Biochem. Biophys. Res. Commun.*, 2000, **275**, 286.

22 B. C. Gilbert, S. Silvester, P. H. Walton and A. C. Whitwood, *J. Chem. Soc., Perkin Trans. 2*, 1999, 1891.

23 C. Jacob, G. L. Giles, N. M. Giles and H. Sies, *Angew. Chem., Int. Ed.*, 2003, **42**, 4742.

24 N. Yasui and T. Koide, *J. Am. Chem. Soc.*, 2003, **125**, 15729.

25 D. D. Perrin, W. L. F. Armarego and D. R. Perrin, *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, 1980.

26 E. Labisbal, J. Romero, A. D. Blas, J. A. Garcia-Vazquez, M. L. Duran, A. Castineiras, A. Sousa and D. E. Fenton, *Polyhedron*, 1992, **11**, 53.

27 B. Bleaney and K. D. Bowers, *Proc. R. Soc. London, Ser. A*, 1952, **214**, 451.

28 O. Kahn, *Molecular Magnetism*, VCH, Weinheim, 1993.

29 (a) A. C. T. North, D. C. Phillips and F. S. Mathews, *Acta Crystallogr., Sect. A*, 1968, **24**, 351; (b) N. Walker and D. Stuart, *Acta Crystallogr., Sect. A*, 1983, **39**, 158.

30 G. M. Sheldrick, *SHELX-97, Program for crystal structure solution and refinement*, University of Göttingen, Göttingen, Germany, 1997.

31 C. K. Johnson, *ORTEP, Report ORNL-5138*, Oak Ridge National Laboratory, Oak Ridge, TN, 1976.

32 J. Bernadou, G. Pratviel, F. Bennis, M. Girardet and B. Meunier, *Biochemistry*, 1989, **28**, 7268.

33 (a) M. S. Lah and V. L. Pecoraro, *Inorg. Chem.*, 1991, **30**, 878; (b) M. S. Lah, B. Gibney, D. Tierney, J. E. Penner-Hahn and V. L. Pecoraro, *J. Am. Chem. Soc.*, 1993, **115**, 5857; (c) J. J. Bodwin, A. D. Cutland, R. Malkani and V. L. Pecoraro, *Coord. Chem. Rev.*, 2001, **216**, 489.

34 A. M. Thomas, M. Nethaji and A. R. Chakravarty, *J. Inorg. Biochem.*, 2004, **98**, 1087.

35 (a) B. Meunier, G. Pratviel and J. Bernadou, *Bull. Soc. Chim. Fr.*, 1994, **131**, 933; (b) B. Armitage, *Chem. Rev.*, 1998, **98**, 1171.

36 C. J. Burrows and J. G. Muller, *Chem. Rev.*, 1998, **98**, 1109.

37 D. W. Tapley, G. R. Buettner and J. M. Shick, *Biol. Bull.*, 1999, **196**, 52.