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DNA cleavage by homo- and heterotetranuclear Cu(II) and Mn(II) complexes with tetrathioether-tetrathiol moiety

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Abstract

Novel homotetranuclear Cu(II) and heteronuclear Cu(II)-Mn(II) complexes with tetrathioether-tetrathiol moiety have been prepared and their DNA relaxation activities with plasmid pCYTEXP (5kb) were electrophoretically established. The cleavage products analyzed by neutral agarose gel electrophoresis indicated that the interaction of the metal complexes with supercoiled plasmid DNA yielded linear, nicked or degraded DNA. The relaxation activities of both homo- and heterotetranuclear (SK4) complexes are time- and concentration-dependent. The findings suggest that SK4 with potent nucleolytic activity is a good nuclease substitute in the presence of cooxidant. Furthermore, the observation of induction of DNA into smaller fragments by SK4 is also significant.

Introduction

Nucleolytic activities of several copper containing redox active coordination complexes have been reported (Sato *et al.* 1994; Routier *et al.* 1996; Sreedhara *et al.* 1999). These nuclease mimics induce DNA cleavage under physiological pH and temperature via metal mediated processes, and their activities involve reversible formation of weak or strong complexes with DNA followed by the scisson reaction (Travers 1993; Sigman *et al.* 1979; Hertzberg & Dervan 1982). The bis(1,10-phenanthroline)-cuprous ion complex was the first synthetic coordination complex shown to possess DNA-relaxation activity (Travers 1993; Pope & Sigman 1984) followed by derivatives of ferrous-EDTA (Sigman *et al.* 1979; Sigman 1986; Schultz *et al.* 1982), various metalloporphyrins (Tullius 1986; Ward *et al.* 1986), cis-diamino dichloro platinium complexes (Groves & Farrell 1989; Sherman & Lippard 1987) and ruthenium complexes of 4,7-diphenyl-1,10-phenanthroline (Barnard *et al.* 1987). Effective clinical use of platinium complexes (Groves & Farrell 1989; Sherman & Lippard 1987; Veal & Rill 1988) in the therapy of human cancer has stimulated studies of interactions of different metal complexes with nucleic acids (Dervan 1992; Pratviel *et al.* 1993; Papavassiliou 1995; Woodson *et al.* 1993; Ross *et al.* 1999; Sargeson 1996; Gravert & Griffin 1993). Therefore, the development of novel metal complexes which interact and cleave nucleic acids and the understanding of their nature of interaction with DNA would provide more effective utilization of metal complexes for diverse purposes such as in molecular biology, pharmacology and gene therapy (Sigman 1986; Ross *et al.* 1999; Mandal *et al.* 1996; Sigman *et al.*; Corey *et al.* 1990) and in the development of anticancer agents.

The present paper reports the nuclease efficiency of homotetranuclear copper(II) and heteronuclear copper(II)-manganese(II) complexes of a novel ligand containing tetrathioether-tetrathiol moiety.

Materials and methods

Chemicals

pCYTEXP was a gift from Dr J.E.G. McCarthy (Biomolecular Sciences, UMIST, UK). Plasmid pCYT-

EXP was grown in *E. coli* JM101 cells in LB media for overnight (Belev *et al.* 1992) and purified by the NucleoSpin plasmid isolation kit (Macherey, Nagel). Other commercial reagents were of reagent quality and used without further purification.

Physical measurements

C, H, N were analyzed microanalytically on a Hewlett Packard 85 CHN analyzer, Cu and Mn were estimated spectrophotometrically. I.r. spectra were recorded on an ATI Unicam Matson 1000 Model FTIR spectrophotometer and u.v.-vis spectra on an ATI Unicam UV2 Model UV/Vis spectrophotometer. Mass spectrum [FAB(positive)] was recorded at TUBITAK (Gebze, Turkey).

Preparation of the ligand (SK1), its dinuclear copper(II) (SK2), and homotetranuclear copper(II) (SK3) complexes

6,6-methylene-bis(5-mercapto-3-thiahexyl)-4,8-dithiaundecane-1,11-dithiol (SK1), dinuclear copper (SK2) and homotetranuclear copper (SK3) complexes (Figure 1) were prepared as reported (Karaböcek *et al.* 1999).

Preparation of heterotetranuclear copper(II)-manganese(II) complex (SK4)

A mixture of the dinuclear copper complex (0.5 mmol, 335 mg), manganese(II) acetate tetrahydrate (1 mmol, 246 mg) and 1,10-phenanthroline (2 mmol, 360 mg) and a stoichiometric amount of sodium perchlorate (140 mg, 1 mmol) in dry acetone (50 cm^3) was boiled under reflux for 12 h. The resulted green-yellow product (SK4) was fitered, washed with ethanol and diethylether and dried over P_4O_{10}. Yield: 0.74 g (40%), m.p. $> 350\,^{\circ}C$. Mass (FAB-positive), $m/z = 1862[M+1]^+$. Anal. calcd. for $C_{66}H_{64}O_{16}N_8S_8Cl_4Cu_2Mn_2$: C, 42.5; H, 3.4; N, 6.0; Cu, 6.8; Mn, 5.9, found: C, 42.35; H, 3.5; N, 6.1; Cu, 6.65; Mn, 6.0. IR (ν; cm^{-1}) 3064-2914 (C-H), 1429 (C=C), 1084 (ClO_4), 622 (S-Cu), 723 (Mn-N).

DNA cleaving activity

The complexes were dissolved in 10 μl of dimethylsulfoxide and then diluted to 1 ml with Milli-Q water. pCYTEXP (5 kbp) was incubated in a reaction mixture (10 μl) containing various concentrations of the ligand (SK1) and individual metal complexes (SK2, SK3, and SK4) in the absence or presence of magnesium monoperoxyphthalate (MMPP) (0.5 mM) in 20 mM Tris-HCl buffer, pH 7.4 at 37 °C. Reactions were initiated with or without the addition of MMPP and terminated by the addition of 5 μl of a terminating agent containing 10 mM β-mercaptoethanol, 20% glycerol, 25 mM EDTA and 0.05% bromophenol blue:xylene cyanol (1:1) after 5 min of incubation as described previously (Mandal *et al.* 1996) with slight modifications. Preliminary experiments have shown that 3–5 min of incubation with metal complexes causes appreciable DNA cleavage. The samples were loaded on 0.7% neutral agarose gel including 0.5 mg/ml ethidium bromide and were subjected to electrophoresis in a horizontal slab gel apparatus. DNA bands were visualized by UV light and photographed. Cleavage of supercoiled plasmid (form I) as a result of treatment of reagents produces nicked (form II) and linear (form III) plasmids. The relaxed plasmid (form II) is electrophoretically less mobile than forms I and III, and this is readily detected. Quantitation of cleavage prod-

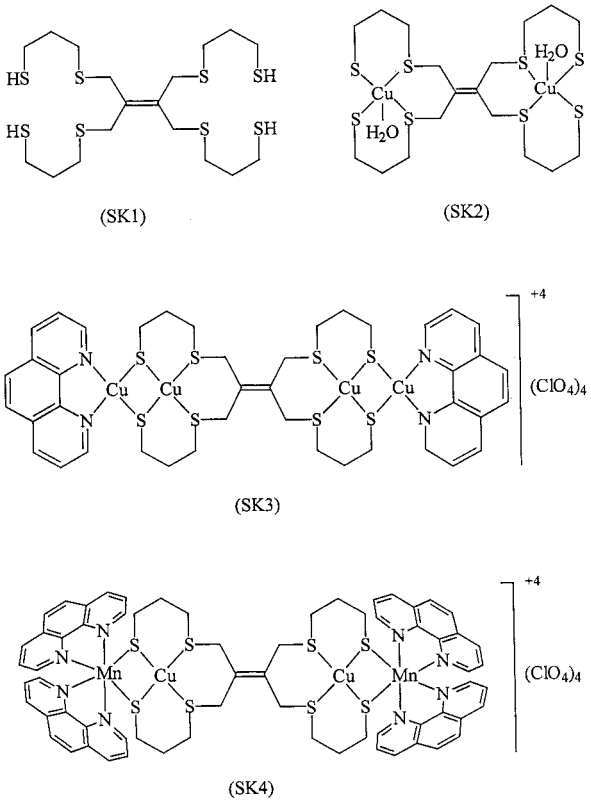

Figure 1. Proposed structures for the ligand (SK1), and its dinuclear copper (SK2), homotetranuclear copper (SK3) and heterotetranuclear copper-manganese (SK4) complexes.

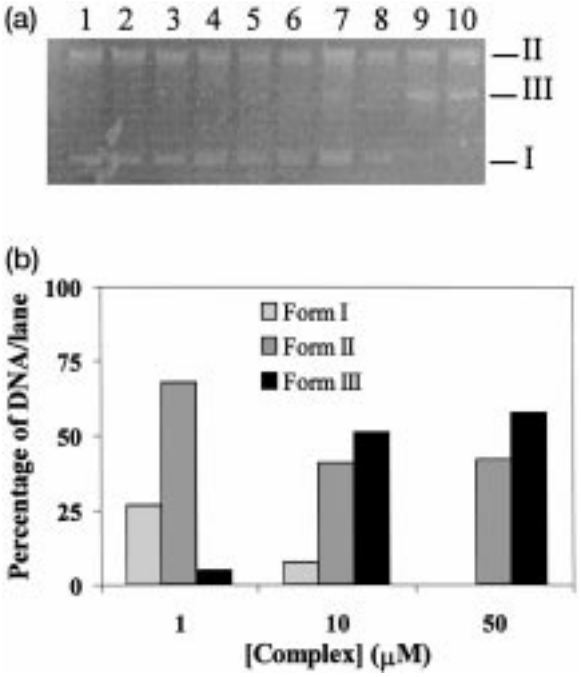

Figure 2. Effect of SK2, SK3 and SK4 on pCYTEXP in the absence of cooxidant MMPP. pCYTEXP was incubated for 5 min as described under Experimental Section. Lanes **1**: 1 μM SK2, **2**: 10 μM SK2, **3**: 50 μM SK2, **4**: 1 μM SK3, **5**: 10 μM SK3, **6**: 50 μM SK3, **7**: Control plasmid, no complex, **8**: 1 μM SK4, **9**: 10 μM SK4, **10**: 50 μM SK4. (**B**) The amount of forms I, II and III of pCYTEXP by SK4 in the absence cooxidant at 5 min of reaction period were determined by Molecular Analyst/Windows software for Bio-Rad's Image Analysis Systems, Version 1.4.

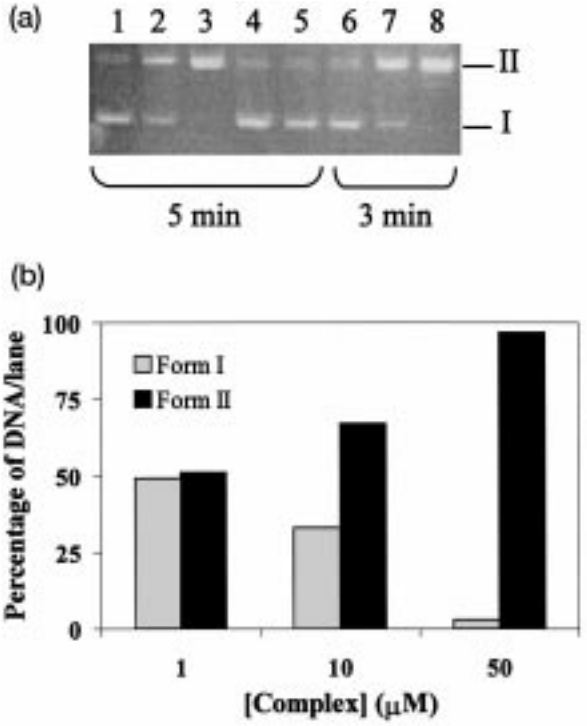

Figure 3. Effect of SK3 on pCYTEXP in the presence of 5 mM cooxidant MMPP. (**A**) pCYTEXP was incubated for 5 min in lanes **1-5**, and for 3 min **6-8** as described under Experimental Section. Lanes **1**: 1 μM SK3, **2**: 10 μM SK3, **3**: 50 μM SK3, **4**: Control plasmid, no MMPP, complex, **5**: no SK3, **6**: 1 μM SK3, **7**: 10 μM SK3, **8**: 50 μM SK3. (**B**) The amount of forms I and II of pCYTEXP by SK3 in the presence of 0.5 mM cooxidant in 3 min of reaction period were determined by Molecular Analyst/Windows software for Bio-Rad's Image Analysis Systems, Version 1.4.

ucts, supercoiled, linear and nicked forms of plasmid DNA, generated as a result of treatment DNA with SK2, SK3 and SK4 was performed by Molecular Analyst/Windows software for Bio-Rad's Image Analysis Systems, Version 1.4.

Results and discussion

The ligand with tetrathioether-tetrathiol moiety (SK1) and its di- and tetranuclear Cu(II), and heterotetranuclear Cu(II)-Mn(II) complexes were prepared and characterized by elemental analyses and spectroscopy. The data in consistent with the earlier reports support the proposed structures of the compounds (Figure 1).

The interactions of the metal complexes (SK2, SK3 and SK4) with DNA in the absence or presence of a cooxidant, magnesium monoperoxyphthalate (MMPP) were investigated using supercoiled form of pCYTEXP (5 kb). Control experiments carried out in the presence of MMPP together with DNA and in the absence of the complexes showed no background cleavage, and chlorates of copper(II) or manganese(II) at concentrations where SK3 and SK4 showed cleavage of DNA were ineffective. In the absence of MMPP, double-strand cleavage of plasmid pCYTEXP was induced only by SK4, and approximately 90% and 100% conversion of the circular supercoiled DNA (form I) to linear DNA (form III) were completed in 5 min at 10 μM and 50 μM concentrations of SK4, respectively, under these conditions (Figures 2A and 2B). This result indicates that only SK4 possess a DNA cleavage activity in the absence of MMPP as a cooxidant.

SK2 had no activity at all while DNA cleaving activity of SK3 occured at higher concentrations when 0.5 mM MMPP was used. Moreover, SK3 possesses a DNA nicking (single-strand) activity especially at 50 μM concentration in the presence of cooxidant (Figure 3A). In the presence of 10 μM of SK3, the

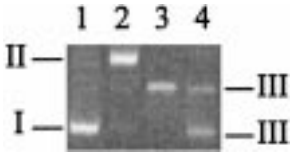

Figure 4. Single strand nicking of SK3 on pCYTEXP. Lane **1** includes only pCYTEXP DNA. pCYTEXP in lane **2** treated with 50 μM SK3 and 5 mM cooxidant MMPP for 5 min as described under Experimental Section. 50 μM SK3 treated pCYTEXP in lane **2** was later digested with only *EcoR I* (lane **3**) or both *EcoR I* and *Cla I* (lane **4**).

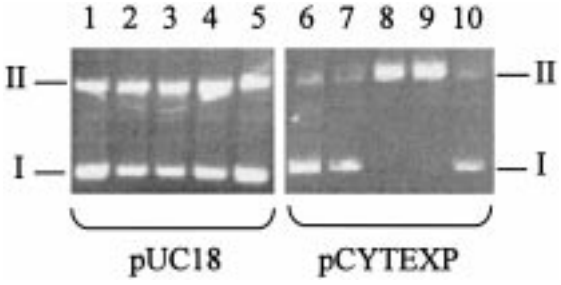

Figure 5. SK3 makes specific single strand nicking. Plasmid DNAs were incubated for 6 min as described under Experimental Section. Lanes **1-5** includes pUC18 DNA, lanes **6-10** includes pCYTEXP DNA. Lanes **1**: only untreated pUC18, **2**: 1 μM SK3 + 5 mM MMPP, **3**: 10 μM SK3 + 5 mM MMPP, **4**: 50 μM SK3 + 5 mM MMPP, **5**: only pUC18 + 5 mM MMPP, **6**: only untreated pCYTEXP, **7**: 1 μM SK3 + 5 mM MMPP, **8**: 10 μM SK3 + 5 mM MMPP, **9**: 50 μM SK3 + 5 mM MMPP, **10**: only pCYTEXP + 5 mM MMPP.

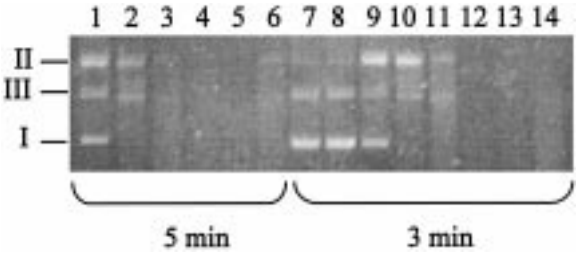

Figure 6. Effect of SK4 on pCYTEXP. pCYTEXP was incubated for 5 min in lanes **1-6**, and for 3 min in lanes **7-14** as described under Experimental Section. All lanes, except lane **8**, include 5 mM MMPP. Lanes **1**: 1 μM, **2**: 5 μM, **3**: 20 μM, **4**: 30 μM, **5**: 40 μM, **6**: 10 μM, **7**: no complex added, **8**: no complex added, **9**: 1 μM, **10**: 5 μM, **11**: 10 μM, **12**: 20μM, **13**: 30 μM, **14**:50 μM SK4.

percent ratio of supercoiled to nicked circle form was (34:66) in 3 min and (28:72) in 5 min, SK3 at 50 μM, however, completely nicked the supercoiled plasmid DNA within 5 min (Figure 3B). These results indicate that nicking activity of SK3 is not only time dependent at lower concentrations (10 μM of SK3) but also concentration dependent. SK3 did not create a new band when *Cla* I and *EcoR* I digested plasmid DNAs (Belev *et al.* 1992) are treated with SK3 in the presence of cooxidant, even if nicks were made in opposing strands, nicks would not occur on nearby sites to produce double strand fragments (Figure 4). None of the digestion produced an extra band showing that SK3 makes only single strand nicking but not double strand.

SK3 makes single strand nicking by producing nicked circles in pCYTEXP and pBR322 (data not shown) but not in pUC18 (Figure 5). Therefore, it can be speculated that SK3 makes site specific nicking in a sequence which is present in pCYTEXP and pBR322 but absent in pUC18. As seen in Figure 5, in the presence of cooxidant, 50 μM of SK3 relaxed supercoiled pCYTEXP by 100% while there was no relaxation on pUC18.

Although the cleavage of DNA by SK4 in the absence of MMPP is appreciable, addition of MMPP increase the cleavage efficiency dramatically. In the presence of MMPP, SK4 at 5 μM and 10 μM concentrations showed very high DNA cleavage activity on pCYTEXP and it cleaved the plasmid DNA at lower concentrations compared to SK3 (Figure 6A). SK4 at 5 μM relaxed supercoiled pCYTEXP DNA by almost 100% only in 3 min at 37 °C and produced nicked circles, and the percent ratio of linear form to nicked circles was greater when concentration was increased to 10 μM. 10 μM of SK4 not only relaxed all supercoiling but also degraded some of the nicked circles in 3 min. Furthermore, SK4 above 10 μM succeded complete degradation of DNA into smaller fragments within 3 min at 37 °C since no ethidium bromide staining was observable.

In summary, novel tetrathioether-tetrathiol containing homo- or heterotetranuclear Cu/Mn complexes were analyzed for their DNA cleavage properties. In the absence of cooxidant, SK4 possesses a concentration-dependent DNA cleavage activity. Addition of cooxidant into the reaction mixture increased the cleaving potency of SK4 and gave SK3 a DNA nicking activity. The difference in the cleavage behaviour of SK4 in the absence/presence of MMPP is consistent with a distinct oxidative cleavage pathway. The relaxation activities of both homo- and heterotetranuclear complexes are time- and concentration-dependent, and time-dependent activity of SK4 is greater than SK3 (Figures 3, 5 and 6). Therefore, SK4 with the greatest nucleolytic activity can be evaluated as a good nuclease substitute in the presence of cooxidant and the observation of induction of DNA

into smaller fragments by SK4 is also significant. New ideas on the molecular design of tetrathioether-tetrathiol based DNA cleaving agents could be extracted from the results described above.

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