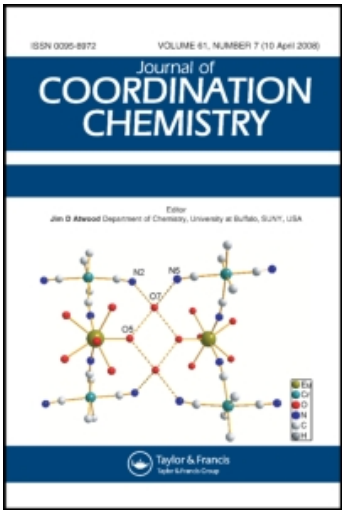

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Ru-edta induced cleavage of DNA

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Ru-edta induced cleavage of DNA

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Ru^{III}-edta (edta, ethylenediaminetetraacetate) induced cleavage of pBluescript SK^+ plasmid DNA in the presence of air with primary oxidant, PO ($PO = H_2O_2$, $KHSO_5$) or reductant (L-ascorbic acid) has been studied at pH 7.2. The studies revealed that the Ru^{III}-edta complex induces DNA cleavage in different ways. A mechanism suggesting the involvement of $[Ru^{V}(edta)O]^-$ in the oxidative cleavage of DNA is proposed for H_2O_2 and $KHSO_5$. Generation of active oxygen radical species ($O_2^{\bullet-}/OH^{\bullet}$) is proposed for cleavage of DNA with Ru^{III}-edta/ascorbate system. Results are discussed in reference to the data reported for the reaction of Ru-edta with DNA constituents, H_2O_2, $KHSO_5$, and L-ascorbic acid.

Keywords: Ruthenium; edta; $KHSO_5$; H_2O_2; Ascorbate; DNA cleavage

1. Introduction

Metal complex mediated cleavage of DNA is a subject of continued interest [1–7], particularly, toward development of new metallo-pharmaceuticals. The importance of ruthenium complexes for promoting such studies in development of ruthenium-based drugs is well established [8–14]. We have been engaged in studying the potential of Ru-pac (pac, polyaminocarboxylate) complexes (figure 1) in various biochemical processes, revealing that Ru-pac complexes are promising with regard to biological applications as DNA binders [10], NO scavengers [10], and protease activity inhibitors [15]. The "pac" ligand is similar in donor character to many proteins of metallo-enzymes that make use of carboxylate and amine donors from amino acids to bind the metal center. As shown earlier [16, 17], and later confirmed by crystallographic studies [18], $edta^{4-}$ (ethylenediaminetetraacetate) is a pentadentate ligand in Ru^{III}-edta (figure 1). The sixth coordination site of the ruthenium center is occupied by an aqua ligand at low pH values or by a hydroxo ligand (figure 1) at high pH values [16, 17]. The $[Ru^{III}(edta)(H_2O)]^-$ complex is labile toward aquo-substitution affording facile and straightforward binding of incoming ligand [16, 17]. We have reported earlier substitution of labile water by purine bases in DNA for direct covalent coordination in ruthenium-DNA adduct formation [10]. Catalytic ability of Ru-edta type complexes toward oxidation of a variety of organic substrates in the presence of

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$R = CH_2COO^-$: pac = $edta^{4-}$

Figure 1. Structural representations of $[Ru(edta)H_2O]^-$.

primary oxidants, PO (PO = H_2O_2, *t*-BuOOH, $KHSO_5$) is well documented in the literature [19]. Recognizing the possibilities of the Ru-edta complex, we plan to study the DNA cleavage activity of Ru-edta. Our goal is to develop a new class of DNA-cleaving agents and to obtain mechanistic insight Ru-edta mediated DNA cleavage. To the best of our knowledge, utilization of Ru-edta as a DNA-cleaving agent in presence of primary oxidant (H_2O_2, $KHSO_5$) or reductant (L-ascorbic acid) has never been documented.

2. Experimental

2.1. *Materials*

[Ru(Hedta)Cl] was prepared by the published procedure [20] and characterized. Anal. Calcd for $C_{11}H_{16}ClKN_2O_9Ru$: C 24.8, 3.94, 5.26; Found: C 24.5, H 3.98, N 5.19. IR, $\nu(cm^{-1})$: 1730 (COOH), 1652 (COO^-). UV–Vis: λ_{max}(nm) ($\varepsilon_{max}(M^{-1}cm^{-1})$) in H_2O: 285 (2650 ± 60), 366 s (630 ± 15). All chemicals were of reagent grade, obtained from Aldrich Chemical Company, and were appropriately degassed before use. Multi-distilled water was used throughout the studies.

2.2. *Instrumentation*

The UV–Vis and IR spectra were collected on Perkin-Elmer Model Lambda 35 and Model 783 spectrophotometers using KBr pellets. A Perkin-Elmer 240C elemental analyzer was used to obtain microanalytical (C, H, N) data.

2.3. *DNA cleavage studies*

Cleavage of supercoiled plasmid pBluescript SK^+ DNA (isolated using Qiagen Plasmid Minikit) by Ru-edta complexes was carried out in 10 μL (TAE) buffer at pH 7.2 (by adopting the procedure reported earlier [21]). The DNA concentration was 10 μM in DNA bp. The buffer, DNA, Ru-pac complex and sufficient water were premixed in an Eppendrof vial, then 2 μL of sodium ascorbate (0.1–0.5 mM) was added and the

reaction was allowed to proceed for 30 min at 37°C before loading onto agarose gel. Agarose gel electrophoresis of plasmid DNA was performed at 50 V 1% slab gels containing 0.5 μg mL^{-1} ethidium bromide in TAE buffer. DNA was visualized by photographing the fluorescence of intercalated ethidium bromide under UV illumination. The cleavage efficiency was determined from the intensities of the bands using a gel documentation system. The super coiled (Form I) and open circular (Form II) forms of plasmid were quantified by densitometric analysis for each treatment using Total Lab Nonlinear Dynamic Image Analysis software (Nonlinear USA Inc., Durham, USA). The conversion from Form I into Form II was represented as percentage plasmid relaxation. Oxidative DNA damage ensuing from the reaction between Ru^{III}-edta and oxidant, PO (PO = H_2O_2, $KHSO_5$) was determined by plasmid relaxation assay as described earlier [22]. The reaction was performed in 200 μL PCR tubes using varied concentrations of PO (0–0.5 mM) in 20 mM phosphate buffer (final volume 25 μL). Plasmid DNA was introduced subsequently and incubated for 1 h at 37°C.

3. Results and discussion

The potential of Ru-edta to cleave DNA was studied by gel electrophoresis using supercoiled pBluescript (SK^+) plasmid DNA (SC) in *tris* buffer. The cleavage of plasmid DNA to Form II (nicked circular, NC) was observed in the presence of Ru-edta, sodium ascorbate and oxygen. Control experiments using only Ru-edta or only sodium ascorbate do not show any appreciable cleavage of DNA (figure 2, lanes 2 and 3). The cleavage efficiency increased with increasing ascorbate concentration (figure 2, lanes 4–6). Based on the above findings and considering the results of electron transfer of Ru-edta with ascorbic acid reported earlier [23], the following working mechanism is proposed (scheme 1) in the cleavage of supercoiled plasmid DNA (SC) by Ru-pac/L-ascorbic acid system.

It had been reported earlier [23] that reduction of Ru(III)-edta by L-ascorbate takes place rapidly (stopped-flow scale) to generate Ru(II)-complex [23]. The oxidation

$$[Ru^{III}(edta)(H_2O/OH)]^{-/2-} + HA^- \longrightarrow [Ru^{II}(edta)(H_2O/OH)]^{2-/3-} + A^{\bullet} \quad (1)$$

$$[Ru^{III}(edta)(H_2O/OH)]^{-/2-} + A^{\bullet -} \longrightarrow [Ru^{II}(edta)(H_2O/OH)]^{2-/3-} + A^- \quad (2)$$

$$[Ru^{II}(edta)(H_2O/OH)]^{2-/3-} + O_2 \longrightarrow [Ru^{III}(edta)(H_2O/OH)]^{-/2-} + O_2^{\bullet -} \quad (3)$$

$$\text{plasmid DNA (SC form)} \xrightarrow{O_2^{\bullet -}} \text{plasmid DNA (nicked/open form)} \quad (4)$$

Scheme 1. Probable mechanism of DNA cleavage by Ru-edta/ascorbic acid/O_2 system.

potential of Ru-edta complexes lie in the range −0.29 to −0.15 V (*vs.* SCE) [19]. Therefore, oxidation of the such Ru(II)-species by molecular oxygen (O_2) is thermodynamically favorable, and perhaps proceeds similar to Fenton's chemistry as proposed in [equation (3)] involving an outer sphere oxidation of the metal ion resulting in formation of corresponding Ru(III)-complex and super oxide radical. The superoxide radical ($O_2^{\bullet -}$), though indiscriminate in oxidative attack, presumably causes single-strand scission by attacking the deoxyribose unit leading to sugar fragmentation, followed by base release and then DNA cleavage [24, 25].

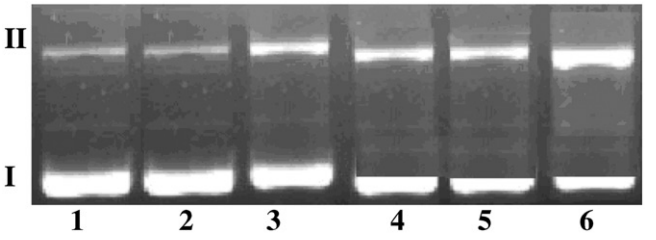

Figure 2. Cleavage of supercoiled pBluescript (SK^+) plasmid DNA by the Ru-edta complex in the presence of sodium ascorbate in TAE buffer at 37°C. Lane 1: The plasmid preparation was good: Form I, II are present, max is Form I. Lane 2: Ru-edta (100 μM) alone caused no DNA nicking. Lane 3: ascorbate (0.5 mM) caused slight DNA nicking, increase in Form II. Lane 4: ascorbate (0.1 mM) + Ru-edta (250 μM); synergistic action; DNA nicking occurred as more of Form II present. Lane 5: ascorbate (0.2 mM) + Ru-edta (250 μM); synergistic action; DNA nicking occurred as more of Form II present. Lane 6: ascorbate (0.5 mM) + Ru-edta (250 μM); increased DNA nicking.

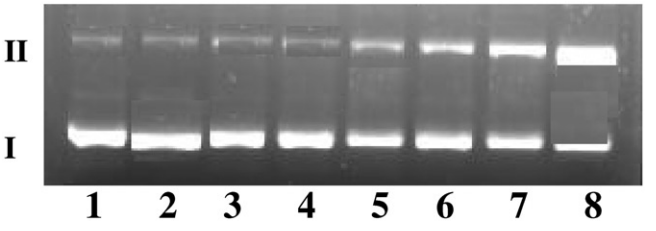

Figure 3. Cleavage of supercoiled pBluescript (SK^+) plasmid DNA by the Ru-edta complex in the presence of sodium ascorbate in TAE buffer at 37°C. Lane 1: The plasmid preparation was good: Form I, II are present, max is Form I. Lane 2: $KHSO_5$ (0.5 mM) caused slight DNA nicking, increase in Form II. Lane 3: H_2O_2 (0.5 mM) alone caused no DNA nicking. Lane 4: Ru-edta (100 μM) alone caused no DNA nicking. Lane 5: $KHSO_5$ (0.1 mM) + Ru-edta (250 μM); synergistic action; DNA nicking occurred as more of Form II present. Lane 6: $KHSO_5$ (0.5 mM) + Ru-edta (250 μM); increased DNA nicking. Lane 7: H_2O_2 (0.1 mM) + Ru-edta (250 μM); synergistic action; DNA nicking occurred as more of Form II present. Lane 8: H_2O_2 (0.5 mM) + Ru-edta (250 μM); increased DNA nicking.

We have also investigated the DNA-cleavage activity of Ru-edta in the presence of primary oxidant, PO (PO = H_2O_2, $KHSO_5$). DNA cleavage efficiency of Ru^{III}-edta was monitored by observing the conversion of supercoiled (Form I) plasmid DNA to the circular nicked form (Form II). Shown in figure 3 are the results of cleavage of supercoiled plasmid DNA. The gel shows the inability of Ru-edta alone to effect cleavage of DNA (figure 3, lanes 2). Results of our control experiments also revealed that, in absence of Ru^{III}-edta, the primary oxidants, $KHSO_5$, and H_2O_2, chosen for this study do not cause significant cleavage of DNA (figure 3, lanes 3 and 4) under specified conditions. However, Ru^{III}-edta shows an appreciable conversion of supercoiled pBluescript (SK^+) DNA (Form I) to nicked circular (Form II) DNA upon prolonged incubation in the presence of either of $KHSO_5$ and H_2O_2. Efficacy of DNA cleavage by Ru-edta increases with increasing amount of PO (figure 2, lanes 5–8). Recently, we reported [26, 27] that oxidation of Ru-edta by PO leads to formation of high-valent Ru(V)-oxo species [18] which is known to oxidize C–H bond in saturated hydrocarbons [18]. Since $[(edta)Ru^{V}=O]^{-}$ was obtained as a predominant product in the reaction of Ru-edta with $KHSO_5$ or H_2O_2 involving heterolytic cleavage of the O–O bond [26, 27], we presume that a mechanism involving high-valent $[(edta)Ru^{V}=O]^{-}$ species similar to

Ru(IV)-oxo polypyridyl complexes [28–30] is operative in the present case. This is supported by almost identical cleavage patterns observed (figure 2, lanes 5–8) for both Ru-edta/$KHSO_5$ and Ru-edta/H_2O_2.

4. Conclusion

The results of present studies clearly demonstrate the nuclease activity of anionic Ru-edta complex on supercoiled DNA in the presence of air with reducing agent (ascorbate) or in the presence of oxidant ($KHSO_5$ or H_2O_2). Although reaction of $[Ru^{III}(edta)(H_2O)]^-$ with AMP was kinetically preferred to other DNA constituents [31, 32], it is difficult to predict the base pair specificity of DNA cleavage by Ru^{III}-edta/ascorbate as it generates freely diffusible radical species. Work pertinent to further probe base pair specificity of DNA cleavage induced by Ru-edta complex needs to be carried out.

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References

[1] S.J. Lippard. *Acc. Chem. Res.*, **11**, 211 (1978).
[2] S.M. Hecht. *Acc. Chem. Res.*, **19**, 383 (1986).
[3] J.K. Bashikin. *Chem. Rev.*, **98**, 937 (1998).
[4] C.J. Burrows, J.G. Muller. *Chem. Rev.*, **98**, 1109 (1998).
[5] C. Metcalfe, J.A. Thomas. *Chem. Soc. Rev.*, **32**, 215 (2003).
[6] K.K. Wing, L. Keith, H.K. Tsang, K.S. Sze, C.K. Chung, T.K.M. Lee, K.Y. Zhang, W.K. Hui, C.K. Li, J.S.Y. Lau, D.C. Ming, N. Zhu. *Coord. Chem. Rev.*, **251**, 2292 (2007).
[7] V.C. da Silveira, J.S. Luz, C.C. Oliveira, I. Graziani, M.R. Ciriolo, A.M.C. da Ferreira. *J. Inorg. Biochem.*, **102**, 1090 (2008).
[8] G. Sava, A. Bergamo. *Int. J. Oncol.*, **17**, 353 (2000).
[9] M.J. Clarke. *Coord. Chem. Rev.*, **232**, 69 (2002).
[10] D. Chatterjee, A. Mitra, G.S. De. *Plat. Met. Rev.*, **50**, 2 (2006) and references therein.
[11] W.H. Ang, P.G. Dyson. *Eur. J. Inorg. Chem.*, 4003 (2006).
[12] K. Karidi, A. Garoufis, A. Tsipis, N. Hadjiliadis, H. den Dulk, J. Reedijk. *Dalton Trans.*, 1176 (2005).
[13] K. Karidi, A. Garoufis, A. Tsipis, N. Hadjiliadis, M. Lutz, A.L. Spek, J. Reedijk. *Inorg. Chem.*, **45**, 10282 (2006).
[14] A. Myari, N. Hadjiliadis, A. Garoufis. *Bioinorg. Chem. Appl.*, **109**, 3 (2005).
[15] D. Chatterjee, A. Mitra, A. Levina, P.A. Lay. *Chem. Commun.*, 2864 (2008).
[16] T. Matsubara, C. Creutz. *Inorg. Chem.*, **18**, 1956 (1979).
[17] H.C. Bajaj, R. van Eldik. *Inorg. Chem.*, **28**, 1980 (1988).
[18] M.M. Taqui Khan, D. Chatterjee, R.R. Merchant, P. Paul, S.H.R. Abidi, D. Srinivas, M.R.H. Siddiqui, M.A. Moiz, M.M. Bhadbhade, K. Venkatasubramanian. *Inorg. Chem.*, **31**, 2711 (1992).
[19] D. Chatterjee. *Coord. Chem. Rev.*, **16**, 273 (1998) and references therein.
[20] A.A. Diamantis, J.V. Dubrwaski. *Inorg. Chem.*, **20**, 1142 (1981).
[21] M.G. Clarke, B. Jansen, K.A. Marx, R. Kruger. *Inorg. Chim. Acta*, **124**, 13 (1986).
[22] K.D. Sugden, K.M. Rigby, B.D. Martin. *Toxicol. Vitro*, **18**, 741 (2004).

[23] D. Chatterjee. *J. Chem. Soc., Dalton. Trans*., 4389 (1996).
[24] R.P. Hertzberg, P.B. Dervan. *J. Am. Chem. Soc*., **104**, 313 (1982).
[25] R.P. Hertzberg, P.B. Dervan. *Biochemistry*, **23**, 3934 (1984).
[26] D. Chatterjee, A. Mitra, R. van Eldik. *Dalton Trans*., 943 (2007).
[27] D. Chatterjee, A. Mitra, R. van Eldik. *Dalton Trans*., 3851 (2008).
[28] N. Grover, H.H. Thorp. *J. Am. Chem. Soc*., **113**, 7030 (1991).
[29] G.A. Neyhart, C-C. Cheng, H.H. Thorp. *J. Am. Chem. Soc*., **117**, 1463 (1995).
[30] B.T. Farrer, H.H. Thorp. *Inorg. Chem*., **39**, 44 (2000).
[31] D. Chatterjee, H.C. Bajaj, A. Das. *J. Chem. Soc., Dalton Trans*., 2497 (1995).
[32] D. Chatterjee, M.S. Ward, R.E. Shepherd. *Inorg. Chim. Acta*, **285**, 170 (1999).