Electrochemical Evidence for the Formation of an Oxygenated Fe–edta Complex

Yuzo Nishida,* Kaori Yoshizawa and Shigeyuki Takahashi

Department of Chemistry, Faculty of Science, Yamagata University, Yamagata 990, Japan

Electrochemical data have revealed evidence for the formation of an oxygenated species of Fe^{II}–edta complex in the presence of oxygen, and this should be a true active species for DNA degradation in the Fe^{II}–edta–O_2 system.

Oxygen activation in metal ion-based biochemical systems has received considerable attention in recent years.[1] Intensively studied ligands include bleomycin (blm),[2] an antitumor antibiotic whose conversion to one or more reactive intermediates involves activation of dioxygen in the presence of metal ions such as Fe^{II} or Cu^{I}. Oxygen activation by Fe^{II}–blm apparently requires an additional electron; this can be provided by the disproportionation of the two Fe^{II}–blm or reductants such as ascorbate, to give an 'activated blm'.[2] However, the detailed nature of the 'activated blm' is not clear at present. It has been reported that the Fe^{II}–edta–O_2 (H_4edta = ethylenediaminetetraacetic acid) system has high activity for DNA degradation.[3] Recently we have observed that the Fe^{III}–edta–ascorbic acid system exhibits high activity for the degradation of DNA, whereas the activity is much lower in the Fe^{III}–dtpa–ascorbic acid system, where H_5dtpa denotes diethylenetriaminepentaacetic acid. In this article we report the first evidence for the formation of an oxygenated species of Fe^{II}–edta in the presence of oxygen, and show that this oxygenated species is a true active species for DNA degradation in the Fe^{II}–edta–O_2 system.

The iron(III) complex with H_5dtpa was isolated as follows: to an aqueous solution (50 ml) containing H_5dtpa (7.87 g) and $NaHCO_3$ (8.4 g) was added 5.41 g of $FeCl_3 \cdot 6H_2O$ with stirring. After one hour, the solution was filtered, and methanol (50 ml) was added to the filtrate. After keeping the solution in the refrigerator for 24 hours, the precipitated yellow crystals were filtered.† The activity for DNA degradation was evaluated by the use of the TBA (2-thiobarbituric

† A satisfactory analysis (C, H, N and Fe) was obtained.

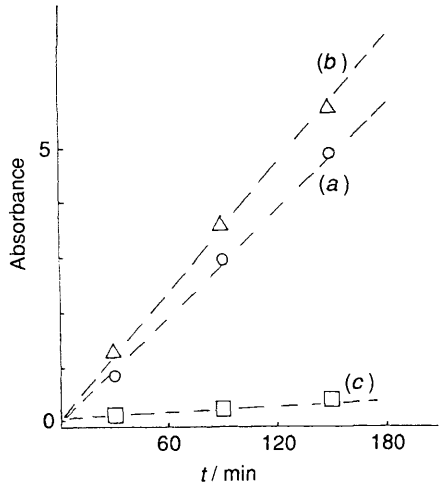


Fig. 1 Time course of absorbance at 532 nm (see text). (*a*) Fe^{III}–edta$^-$;[7] (*b*) $Fe_2O(nta)(MeCO_2)^{3-}$ (H_3nta = nitrilotriacetic acid); (*c*) Fe^{III}–$dtpa^{2-}$.

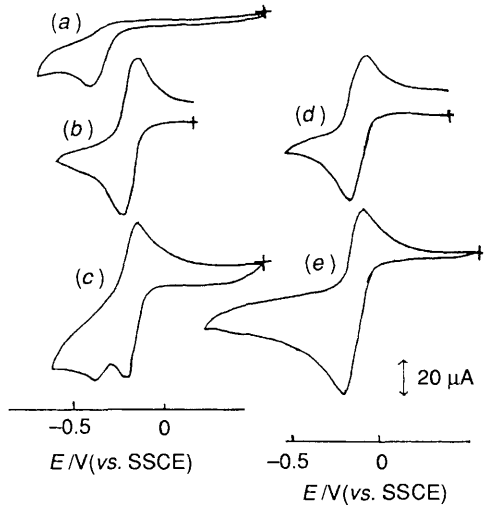
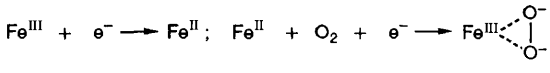


Fig. 2 CV of the metal complexes.‡ (*a*) O_2 (saturated); (*b*) Fe^{III}–$dtpa^{2-}$ (under N_2); (*c*) Fe^{III}–$dtpa^{2-}$ (under O_2); (*d*) Fe^{III}–edta$^-$ (under N_2); (*e*) Fe^{III}–edta$^-$ (under O_2).



Scheme 1

acid)-method.[4] In a typical run, aqueous solutions of DNA (10 ml, 10 mg, calf thymus, Sigma), Fe^{III} complex (10 ml, 0.004 mol dm^{-3}) and ascorbic acid (10 ml, 500 mg) were mixed, and left with stirring at room temperature for 12 h. At the appropriate time after mixing, 4 ml of the reaction mixture was treated with HCl (1 ml; 2 mol dm^{-3}) containing TBA (40 mg), and the resulting solution was heated at 93 °C for 15 minutes. After cooling, the absorbance of the solution at 532 nm was recorded. As shown in Fig. 1, the absorbance at 532 nm increases with time in the case of the Fe^{III}–edta–ascorbic acid system, indicating a high activity of this system for DNA degradation. It should be noted here that the activity of Fe^{III}–dtpa–ascorbic acid system for DNA degradation is negligible.

As illustrated in Fig. 2, the electrochemical properties‡ of the Fe^{III}–edta and Fe^{III}–dtpa complexes are similar under an atmosphere of nitrogen; the Fe^{III} state is reduced to the Fe^{II} state at −0.18 and −0.22 V (*vs.* SSCE)‡ for Fe^{III}–edta and Fe^{III}–dtpa complexes, respectively. However, the presence of dioxygen molecules in solution resulted in a drastic change in the cyclic voltammogram (CV) of the Fe^{III}–edta complex. As shown in Fig. 2, the reduction wave of oxygen is observed at −0.42 V (*vs.* SSCE) under our experimental conditions.‡ The CV property of the Fe^{III} ion in the Fe^{III}–dtpa complex is nearly independent of the presence of dioxygen molecules; *e.g.* both of the reduction waves, $Fe^{III} \rightarrow Fe^{II}$ and $O_2 \rightarrow O_2^-$ are observed separately, and no increase or decrease of the current for reduction or oxidation step was observed in the $Fe^{III} \rightleftharpoons Fe^{II}$ process. On the other hand, in the case of the Fe^{III}–edta complex the CV exhibited increased current for the reduction wave of Fe^{III}/Fe^{II} (−0.18 V) relative to that observed under anaerobic conditions. This increase suggests that the Fe^{II}–edta, once formed, can combine with a dioxygen molecule and undergo further reduction at the electrode surface. An analogous observation has been made for reduced Fe and Mn complexes.[5,6]

‡ The electrochemical measurements were made as follows; in Na_2SO_4 (0.5 mol dm^{-3}), 25 °C, metal complex (0.004 mol dm^{-3}), glassy carbon electrode, scan speed 0.1 V s^{-1}, and the potential was referenced to saturated sodium chloride calomel electrode (SSCE). The CV data for the O_2 molecule and metal complexes in the presence of O_2 were obtained after bubbling O_2 gas through the solution for 20 minutes.

We also observed that the electrochemically reduced, oxygenated Fe^{II}–edta complex exhibits high ability for DNA degradation; 8 ml of an aqueous solution containing Fe^{III}–edta (0.008 mmol) and DNA (8 mg) was electrolysed at −0.2 V (*vs.* SSCE) (passage of electrons, 3.1 C) in a constant flow of air. It was found that the electrochemically activated Fe^{II}–edta complex effects DNA degradation, giving TBA-active substances, however, the activity of the Fe^{III}–dtpa complex for DNA cleavage was negligible under the same experimental conditions.

The foregoing data are consistent with an activation mechanism involving the initial reduction of Fe^{III}–edta, followed by simultaneous binding with O_2 and an electron, giving an iron(III)–peroxide adduct (*cf.* Scheme 1),[7] which may be a true active species for DNA degradation.[8] The negligible activity of Fe^{III}–dtpa may be due to the fact that the iron ion in this complex is surrounded by eight donor atoms, preventing the approach of dioxygen to the iron atom, therefore the oxygenation reaction does not occur.

Received, 22nd August 1991; Com. 1/04417F

References

1 T. G. Spiro, *Metal Ion Activation of Dioxygen*, Wiley, New York, 1983.
2 J. Stubbe and J. W. Kozarich, *Chem. Rev.*, 1987, **87**, 1107.
3 R. P. Hertzberg and P. B. Dervan, *J. Am. Chem. Soc.*, 1982, **104**, 313.
4 E. A. Sauamille, R. W. Stein, J. Peisach and S. B. Horvitz, *Biochemistry*, 1978, **17**, 2746.
5 R. B. Van Atta, E. C. Long, S. M. Hecht, G. A. Marel and J. H. Boom, *J. Am. Chem. Soc.*, 1989, **111**, 2722.
6 S. E. Creager and R. W. Murray, *Inorg. Chem.*, 1987, **26**, 2612.
7 S. Ahmad, J. D. McCallum, A. K. Shiemke, E. H. Appelman, T. M. Loehr and J. S.-Loehr, *Inorg. Chem.*, 1988, **27**, 2230.
8 Y. Nishida and T. Yokomizo, *Inorg. Chim. Acta*, 1989, **163**, 9.