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Vitamin C Causes a Much-Pronounced-Damage to DNA in Presence of Cd^{2+} but not in Presence of Zn^{2+} or Ag^{+}. Why?

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Super oxide dismutase and rhodamine assays carried out in the present study show that free radicals were produced from the interaction between molecular oxygen and 1 : 1 mixture of cadmium(II) acetate and ascorbate or 1 : 1 mixture of silver(I) acetate and ascorbate. Molecular modelling analysis combined with pH measurement shows that in presence of Cd^{2+}, ascorbic acid essentially acts as a strong polyprotic acid whereas in presence of Ag^{+}, it acts as a monoprotic acid. It is believed that the binding of ascorbate with the metal ions Cd^{2+} and Ag^{+} causes the molecular activation which is more susceptible to attack by molecular oxygen. Reactive oxygen species produced from the interaction between activated ascorbate species and molecular oxygen damage DNA.

Key Words: Ascorbate, Cadmium, Activation, Reactive oxygen species, DNA damage, Polydentate ligand, SOD, Xanthine oxidase.

INTRODUCTION

Recently, it has been reported[1] that there is an increased damage to DNA due to ascorbate in presence of Cd^{2+}. When cadmium(II) acetate and ascorbate were present together in 1 : 1 molar ratio, it was found that damage to pBR322 plasmid DNA was about 100 times greater than that due to cadmium(II) acetate or ascorbate alone. Likewise, damage to salmon sperm and calf thymus DNAs by ascorbate was also greatly potentiated due to the presence of cadmium(II) acetate. It was also found that damage to pBR322 plasmid and salmon sperm DNAs was greater in presence of 1:1 mixture of silver(I) acetate and ascorbate than that due ascorbate or silver(I) acetate alone[2]. However, DNA damage caused by 1 : 1 mixture of cadmium(II) acetate and ascorbate was found to be much greater than that due to 1 : 1 mixture of silver(I) acetate and ascorbate. No such potentiation in DNA damage was observed in presence of 1 : 1 mixture of zinc(II) acetate and ascorbate.

It is well known that ascorbic acid (Vitamin C) which usually acts as an antioxidant can become a pro-oxidant in presence of redox active metal ions such as Fe^{3+} and Cu^{2+} that can undergo redox cycling through Fenton reactions[3, 4]. Reactive oxygen species and other free radicals produced can then damage DNA[5, 6].

Since Cd^{2+} is not redox active, the increased DNA damage due to ascorbate in presence of the metal ion appeared to be intriguing. We carried out molecular

modelling analysis using HyperChem-5 combined with pH measurements and assaying of reactive oxygen species to show that this was due to 1 : 1 covalent binding of Cd^{2+} with ascorbate causing its molecular activation. We also addressed the question why the three metal ions differed in their ability to cause activation of the ascorbate.

EXPERIMENTAL

Cadmium(II) acetate, silver(I) acetate, zinc(II) acetate, ascorbic acid, super oxide dismutase (SOD) (EC 1.15.1.1) from Bovine Erythrocytes, (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) (XTT), xanthine, xanthine oxidase (XO) (EC 1.1.3.22) from microorganism, rhodamine B and EDTA were obtained from Sigma-Aldrich, NSW, Australia.

UV-difference spectral analysis

Solutions of cadmium(II) acetate (1 mM) and ascorbate (1 mM) were made in 0.10 M $NaNO_3$ and were mixed in varying proportions[7] at pH 7.4 so that the concentration of each varied from 0 to 1 mM. Similarly, solutions of silver(I) acetate (1 mM), zinc(II) acetate (1 mM), made in 0.10 M $NaNO_3$ were mixed with solutions of ascorbate so that the concentration of each reactant varied from 0 to 1 mM. The reaction mixtures were kept in the dark for 24 h. At the end of the period, each solution was scanned against the corresponding ascorbate blank to obtain its UV-difference spectrum. The absorbance values at λ_{max} or λ_{min} were plotted against added concentration of the reactants to determine the binding ratios of the reactants.

Detection of reactive oxygen species

SOD assay: Decrease in SOD activity determined by xanthine oxidase (XO)/XTT method[8] was used as a measure of the production of super oxide ion. In short, into 2.5 mL of sodium phosphate buffer (50 mM, pH 7.5) were added 0.1 mL each of xanthine (3 mM), EDTA (3 mM), XTT (0.3 mM) and sample solution containing ascorbate plus SOD, cadmium(II) acetate plus SOD, 1 : 1 mixture of cadmium(II) acetate and ascorbate plus SOD, silver(I) acetate plus SOD, 1 : 1 mixture of silver(I) acetate and ascorbate plus SOD or mQ water plus SOD. The reaction was initiated by the addition of XO solution (0.1 mL). The absorbance at 470 nm was monitored for about 10 min using a Cary 1A UV-Vis spectrophotometer at 25°C.

Rhodamine B assay: Rhodamine B assay[9] was used as a measure of the production of reactive oxygen species such as oxygen-based free radical. About 0.60 mg of rhodamine B was dissolved in 100 mL of mQ water. To 1 mL of the rhodamine B solution was added 1 mL of cadmium(II) acetate (0.50 mM), silver(I) acetate (0.50 mM), ascorbate (0.50 mM), 1 : 1 mixture of 0.50 mM cadmium(II) acetate and 0.50 mM ascorbate or 1 : 1 mixture of silver(I) acetate (0.50 mM) and ascorbate (0.50 mM). Absorbance at 533 nm was monitored using a Cary 1A UV-Vis spectrophotometer. Production of free radical would cause an immediate drop of absorbance at 533 nm.

pH measurements: pH of 1 mM solutions of cadmium(II) nitrate, cadmium(II) acetate, silver(I) nitrate, silver(I) acetate, ascorbic acid, 1 : 1 mixtures of cadmium(II) nitrate and ascorbic acid and 1 : 1 mixtures of silver(I) nitrate and ascorbic acid were measured at 25°C immediately after preparation (or mixing) and then periodically up to 24 h (and in some cases after longer periods as well) (Table-1).

TABLE-1
pH CHANGES DUE TO INTERACTION BETWEEN SOLUTIONS OF Cd(II) NITRATE AND ASCORBIC ACID, SILVER(I) NITRATE AND ASCORBIC ACID AT 25°C

Solution	Initial pH or that immediately after mixing	pH after 24 h
1 mM $Cd(NO_3)_2$ in mQ water	5.94	5.94
1 mM ascorbic acid in mQ water	3.80	3.80
1 : 1 mixture of 1 mM $Cd(NO_3)_2$ and 1 mM ascorbic acid (both made in mQ water)	3.68	2.80
1 mM $Cd(NO_3)_2$ in 0.10 M $NaNO_3$	5.72	5.72
1 mM ascorbic acid in 0.10 M $NaNO_3$	3.84	3.84
1 : 1 mixture of 1 mM $Cd(NO_3)_2$ and 1 mM ascorbic acid (both made in 0.10 M $NaNO_3$)	3.65	3.12
100 mM $Cd(NO_3)_2$ in 0.10 M $NaNO_3$	4.08	4.08
100 mM ascorbic acid in 0.10 M $NaNO_3$	2.80	2.80
1 : 1 mixture of 100 mM $Cd(NO_3)_2$ and 100 mM ascorbic acid (both made in 0.10 M $NaNO_3$)	2.45	2.21
1 mM $AgNO_3$ in mQ water	6.42	6.41
1 : 1 mixture of 1 mM $AgNO_3$ and 1 mM ascorbic acid (both made in mQ water)	3.76	3.57
1 mM $AgNO_3$ in 0.10 M $NaNO_3$	6.13	6.12
1 : 1 mixture of 1 mM $AgNO_3$ and 1 mM ascorbic acid (both made in 0.10 M $NaNO_3$)	3.72	3.54

Molecular modelling: Geometry optimizations of ascorbic acid, adducts of ascorbate with Cd^{2+} and Ag^{+} were carried out based on molecular mechanics (using MM^{+} force field) and semi-empirical calculations using HyperChem-5 molecular visualization and simulation program[10]. For geometry optimization using both molecular mechanics and semi-empirical calculations, Polak-Ribiere routine with RMS gradient of 0.01 as the termination condition was used. To simulate the conditions in solution, the molecules were placed in a periodic box of dimensions 50.0 × 50.0 × 50.0 Å containing a maximum of 4130 TIP3P water molecules[11, 12]. The minimum distance between solvent molecules and solute atoms was set at 2.3 Å. Molecular dynamics calculations were carried out to obtain a lower energy minimum by enabling the molecules to cross potential barriers[13]. The parameters used in simulated annealing were: heat time = 1 ps, run time = 0.5 ps, cool time = 0 ps, step size = 0.0005 ps, bath relaxation time = 0.1 ps, starting temperature = 100 K, simulation temperature = 300 K, temperature step = 30 K and data collection period = 4 time steps. For structures optimized based on semi-empirical calculations using ZINDO/1 program, atomic charges were noted.

RESULTS AND DISCUSSION

UV-difference spectral analysis

A plot of absorbance *vs.* concentration at 266 nm (the wavelength at which the absorbance was found to be a minimum) applying to the continuously varying mixtures of cadmium(II) acetate and ascorbate at pH 7.4 showed that Cd^{2+} combined with ascorbate forming mainly 1 : 1 adduct (Fig. 1).

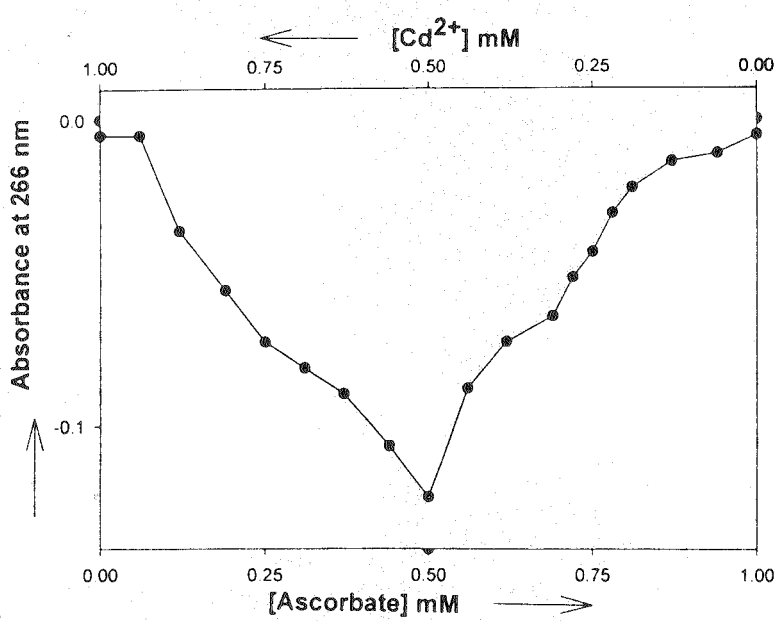


Fig. 1. Absorbance *vs.* concentration plot applying to the continuously varying mixtures of 1 mM cadmium(II) acetate and 1 mM ascorbic acid both made in 0.10 M $NaNO_3$ at pH 7.4 at room temperature at 266 nm showing the formation of 1 : 1 adduct between Cd^{2+} and ascorbic acid

Similarly, a plot of absorbance *vs.* concentration at 232 nm (the wavelength at which the absorbance was found to be a maximum) applying to the continuously varying mixtures of silver(I) acetate and ascorbate at pH 7.4 showed that Ag^+ combined with ascorbate forming mainly 1 : 1 adduct (Fig. 2). No such adduct was

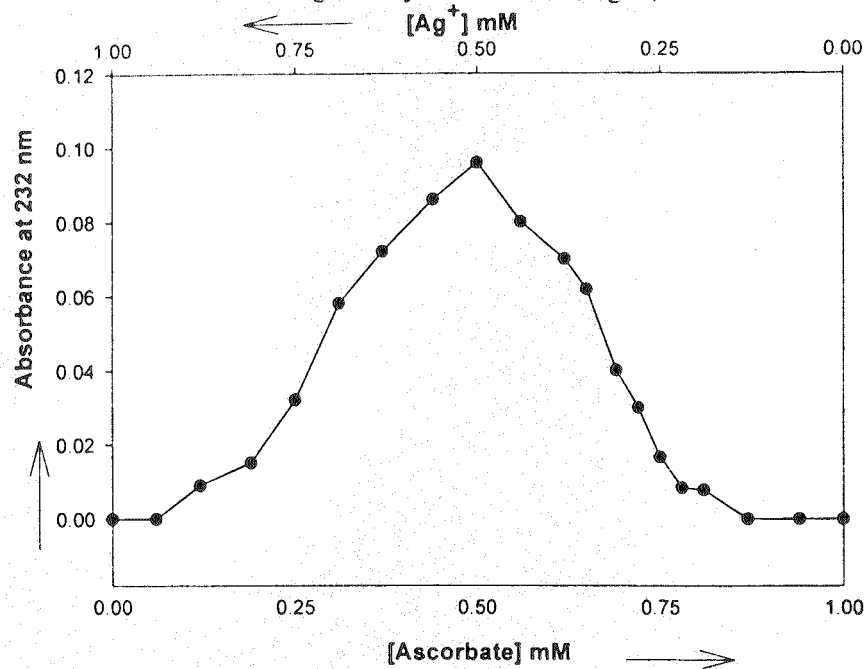


Fig. 2. Absorbance *vs.* concentration plot applying to the continuously varying mixtures of 1 mM silver(I) acetate and 1 mM ascorbic acid both made in 0.10 M $NaNO_3$ at pH 7.4 at room temperature at 232 nm showing the formation of 1 : 1 adduct between Ag^+ and ascorbic acid

found to be formed in the case of continuously varying mixtures of zinc(II) acetate and ascorbate at pH 7.4 (results not shown). [It should, however, be noted that the absence of a clearly defined maximum or minimum in the absorbance *vs.* concentration plot does not preclude the binding of zinc with ascorbate].

SOD and Rhodamine B assay

SOD assay: It was found that absorbance for SOD/XTT/XO at 470 nm was about 0.175. The value was practically the same when, in addition, either cadmium(II) acetate, silver(I) acetate or ascorbate was also present. However, when in addition, 1 : 1 mixture of cadmium(II) acetate and ascorbate was present, there was an immediate decrease in absorbance to 0.0702 which decreased further to reach the minimum value of 0.0598 after 12 min.

When in addition, 1 : 1 mixture of silver(I) acetate and ascorbate was present, immediately after mixing the absorbance was found to be 0.1593 which decreased a little further to reach the minimum value of about 0.1448 after 12 min.

The results show that in both cases the production of superoxide ions is complete within 12 min. A larger decrease in absorbance in the case of 1 : 1 mixture of cadmium(II) acetate and ascorbate in air than that in the case of 1 : 1 mixture of silver(I) acetate and ascorbate in air indicates that more superoxide ions are produced by the former than by the latter. It should however be noted that some metal ions may inhibit XO directly or *via* ascorbate-driven OH* formation and the enzyme is inactive at low pH values.

Rhodamine B assay: For the diluted rhodamine B solution absorbance at 533 nm was 0.620 which immediately decreased to 0.0522 when it was mixed with 1 : 1 mixture of cadmium(II) acetate and ascorbate (both being at 1 mM). As the mixture was left standing, absorbance was found to decrease only slightly to reach the minimum value of about 0.0402 after 10 min.

No such decrease in absorbance was observed when it was mixed with either cadmium(II) acetate, silver(I) acetate or ascorbate. When the rhodamine B solution was mixed with 1 : 1 mixture of silver(I) acetate and ascorbate, absorbance decreased to 0.487 immediately after mixing which further decreased to 0.442 after 15 min.

The results show that more hydroxyl radicals are produced in presence of 1 : 1 mixture of cadmium(II) acetate and ascorbate in air than in presence of 1 : 1 mixture of silver(I) acetate and ascorbate in air.

Molecular modelling analysis and pH measurements

The results of molecular modelling analysis show that when Cd^{2+} was allowed to bind to the deprotonated hydroxyl group attached to C3 position (*i.e.*, the most acidic group in ascorbic acid) and five water molecules (Fig. 3) and the resulting structure optimized, there was a marked increase in polarity of the OH group attached to C2 position (for the free acid, the charges on the H and O atoms were +0.216 and –0.234 units, respectively whereas for the ascorbate ion coordinated to Cd^{2+} these were +0.324 and –0.460 units, respectively).

When solutions of cadmium(II) nitrate and ascorbate were mixed together, it was found there was a decrease in pH immediately after mixing and further

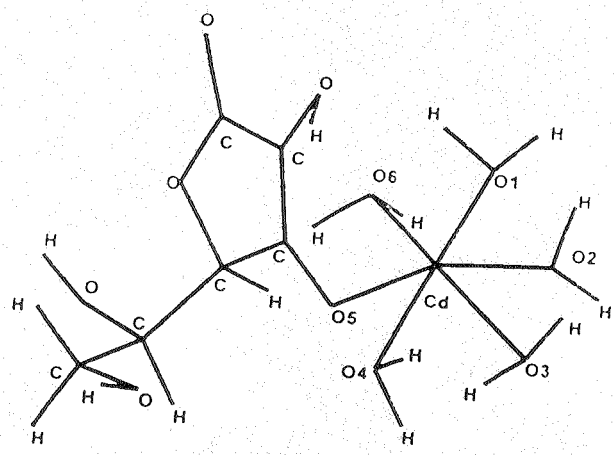


Fig. 3. Proposed structure of 1 : 1 adduct between Cd^{2+} and ascorbate in which the metal ion is considered to be bonded to C3 deprotonated hydroxyl group and five water molecules

decrease in pH occurred over the next 10 h or so (Table-1 and Fig. 4). For example, when equal volumes of 1 mM solution of cadmium(II) nitrate in mQ water (pH 5.94) and 1 mM ascorbic acid in mQ water (pH 3.80) were mixed together, the pH decreased to 3.68 immediately after mixing and after 24 h it reached a value of 2.80. When equal volumes of 1 mM solutions of cadmium(II) nitrate and ascorbic acid in 0.10 M $NaNO_3$ were mixed together, pH dropped to 3.16 after 24 h and to 3.02 after 120 h.

To explain the decrease in pH produced when solutions of cadmium(II) nitrate and ascorbic acid were mixed together, guided by the large increase in bond polarity

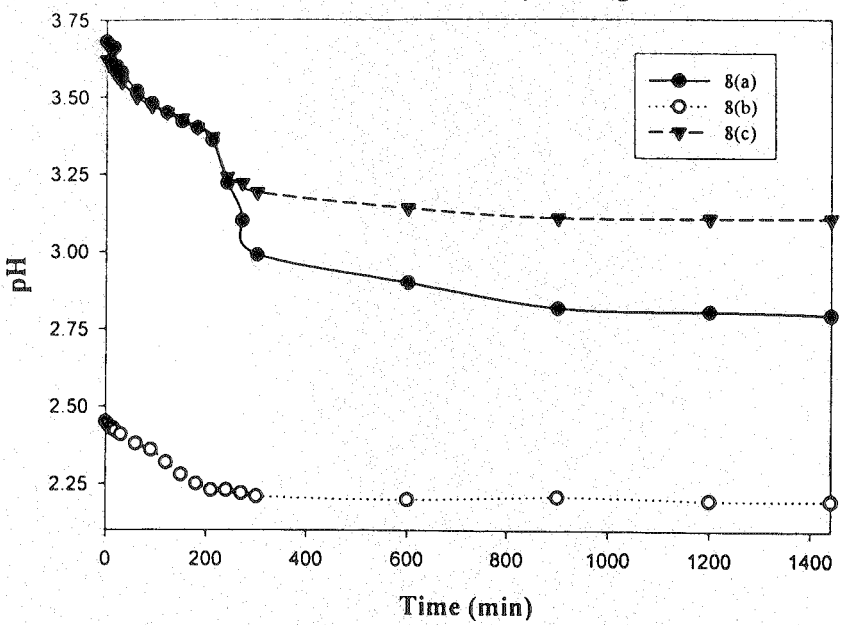


Fig. 4. Changes in pH over time when solutions of cadmium(II) nitrate and ascorbic acid are mixed together: (a) 1 : 1 mixture of 1 mM $Cd(NO_3)_2$ and 1 mM ascorbic acid, both made in mQ water, (b) 1 : 1 mixture of 100 mM $Cd(NO_3)_2$ and 100 mM ascorbic acid, both made in 0.100 M $NaNO_3$, (c) 1 : 1 mixture of 1 mM $Cd(NO_3)_2$ and 1 mM ascorbic acid, both made in 0.1 M $NaNO_3$

seen in molecular modelling calculations, it was concluded that in solutions in 0.10 M NaNOa and at concentrations less than or equal to 1 mM, the hydroxyl group attached to C2 position was also partially deprotonated (by about 38% after 24 h and 89% after 120 h) followed by its coordination to the metal ion (Fig. 5).

When the solutions were made in mQ water instead of 0.10 M $NaNO_3$, it was found that the decrease in pH was even greater such that the complete ionization

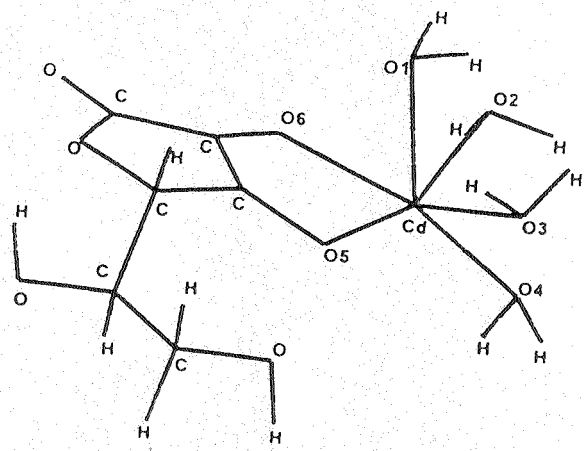


Fig. 5. Proposed structure of 1 : 1 adduct between Cd^{2+} and ascorbate in which the metal ion is considered to be chelated to ascorbate through C3 and C2 deprotonated hydroxyl groups and four water molecules

of the second hydroxyl group as well (*i.e.*, release of two protons altogether from ascorbic acid) was not enough to account for the observed decrease in pH. For example, whereas 1 mM ascorbic acid and 1 mM cadmium(II) nitrate both made in mQ water had a pH of 3.80 and 5.94 respectively, when equal volumes of the two solutions were mixed together, the pH changed to 3.68 immediately after mixing and to 2.80 after 24 h. A third proton was thus made free by allowing the internal ester bond to hydrolyze followed by dissociation of the resulting carboxyl group. The deprotonated carboxyl group also was considered to bind to the metal ion. Thus in the resulting adduct, the trideprotonated ascorbate ion acted as a tridentate ligand being bonded to Cd^{2+} through the two deprotonated hydroxyl oxygens and the deprotonated carboxyl group (Fig. 6). Indeed the decrease in pK_a of organic acids due to complexation with a metal ion has been reported earlier[14, 15]. It has also been reported that the binding of a metal to one coordination site of a heterocyclic ligand lowers the pK_a of another site. For example, based on NMR studies it has been established that binding of platinum to N7 position of guanine lowers pK_a of N1 position by about two units[16].

In all the three adducts, Cd^{2+} has been considered to have an octahedral geometry which is a common coordination geometry for the metal ion[17]. Molecular modelling optimization shows that the adduct with dideprotonated ascorbate ion is most distorted (which is believed to be the result of geometrical constraint imposed by the multidentate ligand). For example, in the adduct with dideprotonated ascorbate ion, the angles around cadmium are: O1CdO2: 73.7°, O2CdO3: 102.3°, O3CdO4: 81.2°, O4CdO5: 125.8°, O5CdO6: 80.4°, O6CdO1: 70.2°, O5CdO2: 159.1°; O6CdO4: 135.2°, OlCdO4: 137.5° and O6CdO3: 143.5°,

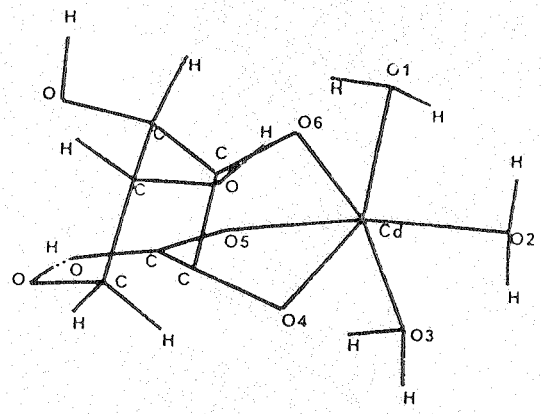
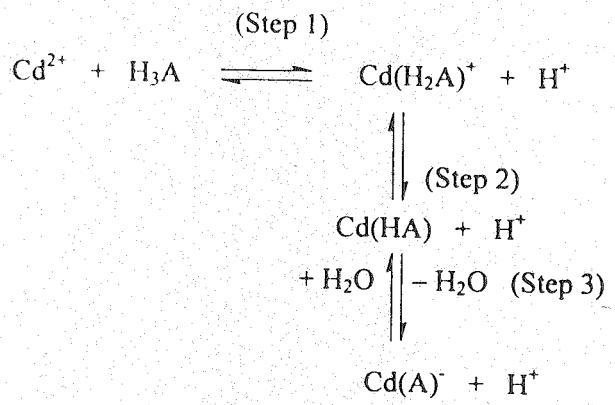


Fig. 6. Proposed structure of 1:1 adduct between Cd^{2+} and ascorbate in which the metal ion is considered to be chelated to ascorbate through C3 and C2 deprotonated hydroxyl groups and four water molecules

showing that the coordination geometry around the metal ion is severely distorted. In the adduct with trideprotonated ascorbate ion, the angles around cadmium are: O1CdO2: 91.7°, O2CdO3: 83.0°, O3CdO4: 97.1°, O4CdO5: 73.9°, O5CdO6: 85.4°, O6CdO1: 83.3°, O5CdO2: 168.5°; O6CdO3: 167.7°, O1CdO4: 152.4°, OSCdO1: 91.7°, O5CdO3: 85.5° and O6CdO2: 72.3°, showing that the distortion from octahedral coordination is much less than the adduct with dideprotonated ascorbate ion.

The schematic of reactions that took place between Cd and ascorbic acid in solution in mQ water is given in **Scheme-1**, where H_3A represents undissociated ascorbic acid, H_2A^- represents monodeprotonated ascorbate ion (commonly



Scheme-1. Schematic reactions between Cd^{2+} and ascorbic acid denoted as H_3A

known as simply the ascorbate), HA^{2-} represents dideprotonated ascorbate ion and A^{3-} represents trideprotonated ascorbate ion obtained by hydration followed by deprotonation of HA^{2-}. The idea of the formation of trideprotonated ascorbate anion and its complexation with a metal ion (in this case Cd^{2+}) is novel. In solution in 0.10 M $NaNO_3$, it is believed that only the first two stages of reactions occurred even after 120 h.

When 1 mM solution of silver(I) acetate in 0.10 M $NaNO_3$ (pH 6.16) and 1 mM solution of ascorbic acid in 0.10 M $NaNO_3$ (pH 3.84) were similarly mixed

together, pH dropped to 3.72 immediately after mixing (Table-1), to 3.54 (after 24 h) and to 3.30 (after 96 h corresponding to 100% ionization of the hydroxyl group attached to C3 position). It was found that as the mixture was left standing at room temperature, there was no further change in pH.

Since pH was kept constant at 7.4 using buffers, the increase in acidity of ascorbic acid solution due to its binding with Cd^{2+} could not be responsible for the observed DNA damage (although this could be relevant in some *in vivo* situations at the cellular level). In addition, the acetate ion from cadmium(II) acetate that was used as the source of Cd^{2+} could also act as a proton acceptor. Hence it is concluded that the most likely cause for the increased DNA damage was the activation of mono- and dideprotonated ascorbate ions due to their binding with Cd^{2+}. It should be noted that both monodeprotonated ascorbate ion and ascorbyl radical (formed from one electron oxidation of monodeprotonated ascorbate ion) have low reduction potentials so that they can be easily oxidized by biologically relevant free radicals and oxidants including ground state triplet molecular oxygen[3]. As stated in the introduction, redox-active metal ions such as those of iron can react with ascorbate and other biological antioxidants such as glutathione, producing free radicals and other reactive oxygen species that can damage DNA. However, the results of the present study are different since cadmium is not redox active under the conditions of the experiments and the conditions that exist in biological systems.

It is suggested that the activated mono- and dideprotonated ascorbate ions bind with ground state triplet molecular oxygen ($^3O_2$), producing highly reactive singlet molecular oxygen ($^1O_2$) that causes direct damage to DNA. The singlet molecular oxygen can also react with water[18] to produce H_2O_2, that damages DNA. The idea of ascorbate activation would also explain why there was no observed DNA damage due to ascorbate in presence of zinc(II) acetate since Zn^{2+} did not appear to bind with ascorbate. A smaller DNA damage observed in presence of 1 : 1 mixture of silver(I) acetate and ascorbate could also be explained in terms of a weaker activation of ascorbate by Ag^{+}. It was found that when Ag^{+} was allowed to bind to oxygen at C3 position and a water molecule and the resulting structure optimized, the polarity of the OH group at C2 position remained essentially unchanged (changes in atomic charges were from +0.216 to +0.225 for H and from –0.234 to –0.232 for O). Hence it is concluded that, unlike that in the case of Cd^{2+}, further deprotonation of monodeprotonated ascorbate ion did not occur in presence of Ag^{+} in line with the observation that the decrease in pH was much less when solutions of silver(I) nitrate and ascorbic acid were mixed together than when solutions of cadmium(II) nitrate and ascorbic acid were mixed together. Thus, whereas ascorbate acted as a polydentate ligand when coordinated to Cd^{2+}, it acted as a monodentate ligand when coordinated to Ag^{+}. No change in pH with time was observed for the 1 : 1 mixture of zinc(II) nitrate and ascorbic acid in line with the observation that Zn^{2+} did not bind with ascorbate.

As the concentration of ascorbic acid and cadmium(II) nitrate was increased above 1 mM, it was found that although there was a progressive decrease in pH with the increase in concentration, the changes in pH due to their interaction became gradually smaller (Fig. 4 (b)). It was found that when no buffer was used to control

pH, at 100 mM concentration even the first stage of dissociation was not required to be completed to account for the observed change in pH. This was not unexpected since the position of each of the three equilibria in the proposed reaction scheme would be shifted to the left as the concentration of H^+ ion was increased. It could be that bi- and tridentate adducts did not form at all at low pH produced in unbuffered solutions (*e.g.*, a pH of 2.22 was produced 24 h after mixing of 0.10 M solutions of ascorbic acid and cadmium(II) nitrate in mQ water).

Finally, it should be mentioned that in this work molecular dynamics calculations were utilized simply to enable the molecules to cross potential barriers so that they reached a lower energy minimum.

Conclusion

It is the binding of ascorbate with Cd^{2+} and Ag^+ that activates ascorbate ions such that they are more susceptible to attack by molecular oxygen. Reactive oxygen species produced in the reaction between activated ascorbate species and the metal ions Cd^{2+} and Ag^+ damage DNA. Zn^{2+} does not appear to cause such molecular activation of ascorbate.

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REFERENCES

1. Z. Hossain and F. Huq, *J. Inorg. Biochem.*, **90**, 85 (2002).
2. ———, *J. Inorg. Biochem.*, **91**, 398 (2002).
3. A. Carr and B. Frei, *FASEB J.*, **13**, 1007 (1999).
4. H.R. Griffiths and J. Lunec, *Toxicol. Pharmacol.*, **10**, 173 (2001).
5. B. Halliwell, *Free Rad. Res.*, **25**, 439 (1996).
6. G.R. Buettner and B.A. Jurkiewicz, *Radial Res.*, **145**, 532 (1996).
7. S.F.A. Kettle, Physical Inorganic Chemistry, A: Coordnation Chemistry Approach, Oxford University Press, p. 79 (1998).
8. H. Ukeda, S. Maeda, T. Ishii and M. Sawmura, *Anal. Biochem.*, **251**, 206 (1997).
9. J. Tanaka and S.L. Suib, Experimental Inorganic Chemistry, Prentice-Hall, Inc., p. 294 (1999).
10. HyperCube HyperChem, Release 5 for Windows, 5.0 ed.; HyperCube, Ed. (1996).
11. J. Ridley and M.C. Zerner, *Theoret. Chim. Acta*, **42**, 223 (1976).
12. F. Huq and M.C.R. Peter, *J. Inorg. Biochem.*, **78**, 217 (2000).
13. W.L. Jorgensen, J. Chandrasekhar, J.D. Madura, R.W. Impey and M.L. Klein, *J. Chem. Phys.*, **79**, 926 (1983).
14. A. Avdeef, A.J. Zelazowski and J.S. Garvey, *Inorg. Chem.*, **24**, 1928 (1985).
15. M. Enamullah, *J. Bangladesh Chem. Soc.*, **10**, 147 (1997).
16. J.K. Barton, in: I. Batinin, H.B. Gray, S.J. Lippard and J.S. Valentine (Eds.), Bioinorganic Chemistry, University Science Press, p. 455 (1994).
17. F.A. Cotton and G. Wilkinson, Advanced Inorganic Chemistry. Wiley, p. 598 (1988).
18. A.D. Wentworth, L..H Jones, P. Wentworth (Jr.), K.D. Janda and R.A. Lerner, *Proc. Nat. Acad. Sci. USA*, **97**, 10930 (2000).

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