Kinetics and Mechanism of Conversion of Carcinogen Hexavalent Cr(VI) to Cr(III) by Reduction with Ascorbate

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Summary: To asses the therapeutic efficacy of ascorbic acid in preventing toxic and mutagenic effects of Cr(VI) to Cr(III) the Kinetics and mechanism of reduction of hexavalent chromium by ascorbate has been studied. The reduction was done under pseudo first order condition by taking an excess of ascorbate over hexavalent chromium species. The rise of absorbance of Cr(III) was monitored at 578 nm. The observed rate constants then determined showed a dependence on both ascorbate concentration and pH as well. The bearing of these observations on the mechanism of reduction of hexavalent chromium is discussed in order to explain this.

Introduction

Various minerals play a vital role in the essential physiological processes. Chromium is one among the such elements and is found to be toxic and mutagenic. Toxicity and mutagenecity of hexavalent chromium [Cr(VI)] compounds has been well documented [1-3]. These compounds are also irritants of the skin and mucous membrane [4,5]. It has been suggested that Cr(VI) crosses the cell membrane and then causes the oxidation of a number of essential cellular components, thereby impairing their function. This cellular damage may also include interference with the genetic machinery making Cr(VI) compounds mutageneic [6,7]. To reduce the damage caused by hexavalent Cr, the living systems may utilize a number of possible mechanisms. The harmful effects of Cr are confined largely to its +6 oxidation state and there is no evidence that Cr(III) has any toxic effects. It is now well known that Cr in +3 oxidation state is an essential trace element having a role in glucose tolerance [8]. It, therefore, seems reasonable that cells may utilize a reduction mechanism to convert toxic Cr(VI) into harmless Cr(III). A number of sub-cellular organelles have been tested and attempts to purify protein were made but these did not yield positive results [9]. On the other hand a number of small molecules of cellular origin are redox active and these may be good candidates for countering chromate toxicity. One such small molecule is ascorbic acid which can reduce Cr(VI) efficiently. Current research have shown that mutagenic and toxic effects of Cr(VI) were lower in rats which were fed high dose of Vitamin C, compared to those which were Vitamin C deficient [10]. In recent years some studies on reduction of Cr(VI) by small molecule reductants have appeared in literature [11-13]. These studies have shown that a variety of mechanisms may be utilized, some having possible biological significance. The present study was initiated to elucidate the mechanism of Cr(VI) reduction by ascorbate [14].

Another problem with Cr(III) is that hydrolysis of this metal decreases the solubility and therefore biological availability of this metal is hindered. To overcome this problem a good chelating agent which can solubilize $Cr(OH)_3$ is required. Ascorbic acid is not only a good reducing agent in the biological environment but also may behave a good chelating agent to solubilize the trace metal to play vital role.

Results and Discussion

Results of the kinetic experiments on reduction of Cr(VI) by ascorbate are summarized in Table-1. These experiments were carried out at a constant temperature of 28°C. Pseudo-first order conditions were maintained by maintaining a very large excess of the reducing agent over Cr(VI).The spectrophotometric study also shows that after reduction of Cr(VI) the oxidized ascorbate may also behave as a chelating agent. The change in $\lambda_{max.}$ value after reduction does not resembles the λ_{max} of from Cr(III) ion but much nearer to the

complexed Cr(III). Results are summarized in Table-2.

Table-1: Summary of the k_{obsd} at the various pH and ascorbate concentration.

$[K_2Cr_2O_7] = 0.001M$

S.No.	[Asc.]	pH=3	pH=4	pH=5
1	0.025	$9.45x10^{-4}$	$7.40x10^{-4}$	$6.43x10^{-4}$
2	0.050	$2.78x10^{-3}$	$1.26x10^{-3}$	$1.16x10^{-3}$
3	0.060	$3.30x10^{-3}$	$1.60x10^{-3}$	$1.21x10^{-3}$
4	0.075	$4.50x10^{-3}$	$2.10x10^{-3}$	$1.41x10^{-3}$
5	0.100	$4.71x10^{-3}$	$2.28x10^{-3}$	$1.45x10^{-3}$

Table-2: Summary of change in $\lambda_{max.}$ when the oxidized ascorbate is chelating agent.

S.No.	Species	$\lambda_{max.}$nm
1	$Cr(NO_3)_3$	588
2	$CrCl_3$	588
3	$Cr(NO_3)_3$ + Ascorbic acid	588
4	Cr(III) (Gly.)	532
5	Cr(III) (Gl.) + Ascorbic acid	577
6	Cr(VI) + Ascorbic Acid	578

It is seen that plots of kobs. vs. [Asc]. at each pH give a reasonable straight line with very nearly zero intercept. This is indicative of first order behaviour in Ascorbate concentration. The recent study by Dixon *et al.* [13] also show a small intercept in k_{obsd} vs [ASC.] plots. In that study CrO_4^{2-} was reduced by ascorbate at a higher pH and both the intercepts as well as slopes were found to decrease with increasing pH. Rate constants for ascorbate reductions of Cr(V) and Cr(IV) [15-17] are orders of magnitude larger than the corresponding rate constants for reductions of Cr(VI) ($Cr_2O_7^{2-}$) at low pH in present study and CrO_4^{2-} at pH (in ref. 13). Over all reaction, therefore must involve a single electron reduction of Cr(VI) as the rate determining step even though each Cr(VI) gains 3 electrons and ascorbate loses two electrons. The trend in pH dependence of the slope of $k_{obs.}$ vs. [ASC] is similar to that reported in ref. 13 i.e., it is minimum at the highest pH and maximum at the lowest pH. On one hand the formal redox potential of ascorbate increases with pH on the other hand dichromate and chromate are in equilibrium.

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} \qquad K = 10^{-5.9}$$

$$H_2CrO_4 \rightleftharpoons HCrO_4^{2-} + H^+ \qquad K = 4.1$$

$$Cr_2O_7^{2-} + H_2O \rightleftharpoons 2HCrO_4^- + CrO_4^- \qquad K = 10^{-2.2}$$

and

$$Cr_2O_7^{2-} + OH^- \rightleftharpoons HCrO_4^- + CrO_4^{2-}$$

Thus basic conditions favour CrO_4^- and $HCrO_4^-$ over $Cr_2O_7^{2-}$ which predominate under acid conditions.

Acid solutions of dichromate are strong oxidants

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \longrightarrow 2Cr^{3+} + 7H_2O \quad E^\circ = 1.33V$$

However, CrO_4^{2-}, under basic conditions is a much poorer oxidant.

$$CrO_4^{2-} + 4H_2O + 3e^- \longrightarrow Cr(OH)_3(s) + 5OH^- \quad E^\circ = -0.13V$$

Since the net reaction driving force is given by

$$E^\circ_{cell} = E^\circ_{oxd.} - E^\circ_{red.}$$

And the rates of outer sphere electron transfer reactions are governed by Marcus relationship

$$k_{12} = \sqrt{k_{11}\, k_{22}\, K_{12}\, f}$$

Where k_{12} is the rate constant of the cross reaction and k_{11} and k_{22} are the self exchange rate constants of oxidant and reductant and K_{12} is cross reaction equilibrium constant.

$$\log f = \frac{(\log K_{12})}{4 \log (k_{11}\, K_{2z}/Z^2)}$$

Thus assuming that self exchange rate constants do not change with pH, the increase in rate constant with pH must be due to an increase in equilibrium constant of Cr(VI)/Ascorbate reaction. This is turn must be due to an increase in E° of the overall reaction since

$$nF\, E^\circ = R\, T \ln k$$

The observed decrease in rate constant as pH is increased implies that the decrease in oxidizing ability of Cr(VI) in going from acidic to basic medium is not compensated by a concomitant increase in reducing ability of ascorbate over the

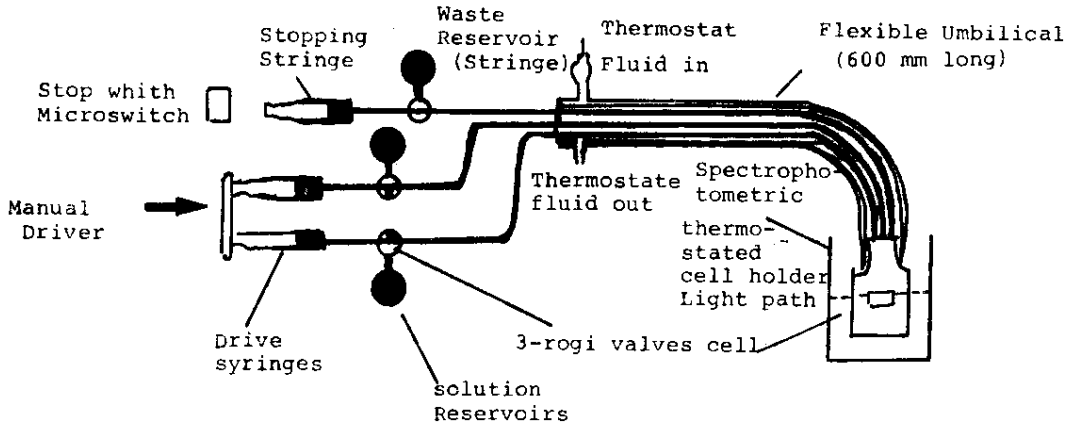

Fig. 1: Schematic representation of SFA-11 stopped flow.

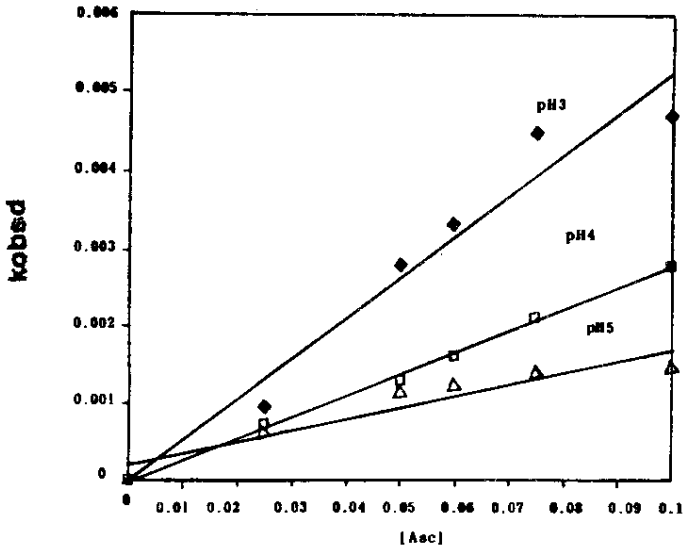

Fig. 2: kobsd. at different pH and [ASC].

similar pH change. The exact quantitative prediction of these changes must await more extensive experiments.

On the other hand some authors have proposed a chromate-ascorbate ester intermediate may facilitate inner sphere electron transfer. The formation of this intermediate is favoured by low pH as an undissociated ascorbic acid can lose a proton to oxygen co-ordinated to Cr(VI) increasing liability of Cr-O bond. However, a direct physical evidence for the formation of chromate-ascorbate ester has not been forthcoming.

We have not attempted a numerical analysis of the quantitative dependence of the slope and intercept in k_{obsd} vs [Asc] plots. These would provide information about all the possible interactions between various possible ascorbate and Cr(VI) species. The overall rate of formation of Cr(III) from Cr(VI) by ascorbate is very slow as compared to reactions of Cr(V) and Cr(VI) which involve free radicals.

Experimental

Solution $K_2Cr_2O_7$ ($1x10^{-3}$M) and ascorbic acid were prepared in formate buffer (pH=3) and in

acetate buffer (pH=4 & 5). Reactions were measured by monitoring increase in absorbance at 578 nm [due to formation of Cr(III)] as a function of time. Reaction were carried out under pseudo first order conditions with ascorbate in very large excess over Cr(VI). Temperature (28°C) was controlled by circulating water into the SFA-11 Stopped-flow from a thermostatic water bath. Reagents were mixed in a High-Tech SFA-11 Stopped-flow (Fig. 1). The observation cuvet was placed in a spectronic 21 spectrophotometer, whose analog out put was digitized with the help of a 12-bit A/D converter (Vernier software Portland. Ore). The digitized voltages were read into an IBM-PC Compatible computer. The voltage vs. time files were then analyzed with the help of an in house software. This software first converted the voltage into the transmittance and then into the absorbance. The linear regression based data-reduction routine then fitted the data to the formula

$$\ln (A_t - A_\alpha) = k\,t + C$$

Where k is pseudo-first order rate constant A_t and A_α are absorbance at time "t" and infinite time "t_α" respectively.

References

1. S.K. Tandon, Biological and Environmental aspects of Chromium, Langard, S. Ed., Elsevier Biomedical Press, Amsterdam (1982).
2. R.D. Hayes, "Biological and Environmental aspects of Chromium" Langard, S. Ed., Elsevier Biomedical Press, Amsterdam, pp. 241-247 (1982).
3. T. Norseth, *Environ. Health perspect.*, **40**, 121-130 (1981).
4. W. Mertz, *Physiol. Rev.*, 49, 163 (1969).
5. W. Mertz and K. Schwartz, *Arch. Biochem. Biophys.*, **58**, 504 (1955).
6. K.W. Jennette, *Biol. Trace Elem. Res.*, **1**, 55-62 (1979).
7. P.H. Connet and K.E. Wtterhann, *Structure and Bonding*, **54**, 93-124 (1983).
8. S. Han, *Biocheng Chemical Abstract*, **108**, 3-4 p. 19673 (1988).
9. J.E. Gruber and K.W. Jennette, *Biochem. Biophys. Res. Commun.*, 700-706 (1978).
10. E. Ginter, D. Chorvatvicova, A. Kosinova, *J. Vit. Nutr. Res.*, **50**, 161-166 (1988).
11. A. McAuley, M. Adegboyega Olatunji, *Can. J. Chem.*, **55**, 3328 (1977).
12. A. McAuley and M. Adegboyega Olatunji, *Can. J. Chem.*, **55**, 3335 (1977).
13. D.A. Dixon, N.P. Sadler and Tara P. Dasgupta, *J. Chem. Soc. Dalton Trans* (1993).
14. Muti-ur-Rahman and S. Arif Kazmi, Abstr. of 3rd National Chem. Conference (pp-73) Dec. 21-24, 1991, Gomal Univ., D.I. Khan Pakistan
15. S.K. Ghosh and E.S. Gould, *Inorg. Chem.*, **28**, 1538 (1989).
16. S.K. Ghosh andf E.S. Gould, *Inorg. Chem.*, **28**, 1948 (1989).
17. S.K. Ghosh, R.N. Bose and E.S. Gould, *Inorg. Chem.*, **26**, 2684 (1987).