

Biologically significant scavenging of the myeloperoxidase-derived oxidant hypochlorous acid by ascorbic acid

Implications for antioxidant protection in the inflamed rheumatoid joint

Barry Halliwell, Mohammed Wasil and Martin Grootveld

Department of Biochemistry, King's College (KQC), Strand Campus, London WC2R 2LS, England

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Ascorbic acid, at physiological concentrations, can scavenge the myeloperoxidase-derived oxidant hypochlorous acid at rates sufficient to protect α_1 -antiprotease against inactivation by this molecule. The rapid depletion of ascorbic acid at sites of inflammation, as in the inflamed rheumatoid joint, may therefore facilitate proteolytic damage.

Ascorbic acid; Myeloperoxidase; Hypochlorous acid; Superoxide; Hydroxyl radical; Neutrophil

1. INTRODUCTION

Ascorbic acid (vitamin C) has complex effects on systems generating oxygen free radicals. For example, ascorbic acid reacts quickly with superoxide radical, O_2^- , and with hydroxyl radical, $\cdot OH$ [1,2] and its ability to scavenge these species may be biologically important in lung [3], at sites of inflammation [4] and in chloroplasts [5]. However, mixtures of ascorbic acid and iron or copper salts can also generate damaging radical species, including $\cdot OH$ [6–8]. Neutrophils are rich in ascorbic acid, much of which becomes oxidized during activation of the respiratory burst [9]. Activated neutrophils also oxidize extracellular ascorbic acid, a reaction that seems to be largely mediated by O_2^- [4]. In the synovial fluid from inflamed rheumatoid joints, both the total ascorbic acid (reduced plus oxidized) and the [ascor-

bate]/[dehydroascorbate] ratio are markedly decreased [10,11]. Even in rheumatoid sera, these values are sub-normal [10,11]. A lowered concentration of ascorbic acid will minimize O_2^- -scavenging antioxidant protection in the inflamed rheumatoid joint [10].

Activated neutrophils release the enzyme myeloperoxidase, which uses some of the H_2O_2 produced during the respiratory burst to oxidise Cl^- into a powerful oxidant that has been identified as hypochlorous acid, $HOCl$ [12–14]. Hypochlorous acid oxidizes a wide range of biomolecules, but its most important cellular target is probably the α_1 -antiprotease protein, which protects tissues against proteases such as elastase, also released from activated neutrophils [12]. Ascorbic acid has already been suggested to react with $HOCl$ [4,14], but it is important to know if this reaction is swift enough at physiological ascorbate concentrations (normal serum and synovial fluid concentrations are in the range 40–141 μM [10,11,15,16]) to protect important biological targets such as α_1 -antiprotease. In the present paper, this point has been investigated.

Correspondence address: B. Halliwell, Dept of Biochemistry, King's College, Strand Campus, London WC2R 2LS, England

2. MATERIALS AND METHODS

2.1. Reagents

α_1 -Antiprotease (type A9024) was from Sigma. Other reagents, including porcine pancreatic elastase, NaOCl and ascorbic acid, were from BDH.

2.2. Assays

Elastase and α_1 -antiprotease were assayed essentially as described in [17,18]; full details are given in the legend to table 1. HOCl was obtained immediately before use by adjusting NaOCl to pH

6.2 with dilute H_2SO_4 , and its concentration determined as in [17]. The HPLC equipment used is described in [18–20].

3. RESULTS

Table 1 (column A) shows that ascorbic acid had no action on elastase activity at the concentrations tested. α_1 -Antiprotease inhibits elastase, and its ability to do this was also unaffected by ascorbic acid (column B). The concentration of α_1 -antiprotease that could inhibit elastase by about 97% was chosen for these experiments, but very similar results were obtained at other elastase: α_1 -antiprotease ratios.

Table 1

Effect of ascorbic acid on elastase, its inhibition by α_1 -antiprotease and inactivation of α_1 -antiprotease by HOCl

Final concentration of ascorbic acid in reaction system A (μM)	Elastase activity (% maximum rate)			
	A	B	C	D
0	100	3	99	98
5	100	3	90	42
10	99	2	81	8
21	101	4	65	3
42	98	3	34	2
83	103	3	15	3
167	100	2	6	2

α_1 -Antiprotease (final concentration 0.5 mg/ml) was mixed with the final concentration of ascorbic acid stated plus HOCl (30 μM final concentration) in buffer at pH 7.4 [17,18] and incubated at 25°C for 60 min (reaction system A). 3 ml of buffer [17,18] are then added, plus elastase. After further incubation for 30 min the elastase activity remaining is assayed as the rise in absorbance at 410 nm [17,18]. Results are expressed as the % of maximum elastase activity (A_{410} 0.0599 $\cdot \text{min}^{-1}$ corresponds to 100%). Column A, both HOCl and antiprotease omitted from reaction system A, so that any effect of ascorbic acid on the elastase activity can be tested. Column B, HOCl omitted from reaction system A, so that any effect of ascorbic acid on the ability of α_1 -antiprotease to inhibit elastase can be tested. Column C, complete reaction mixture as above. Column D, as for C, but HOCl and ascorbic acid pre-incubated for 5 min before adding α_1 -antiprotease, then incubation for a further 60 min before adding elastase and buffer as described above

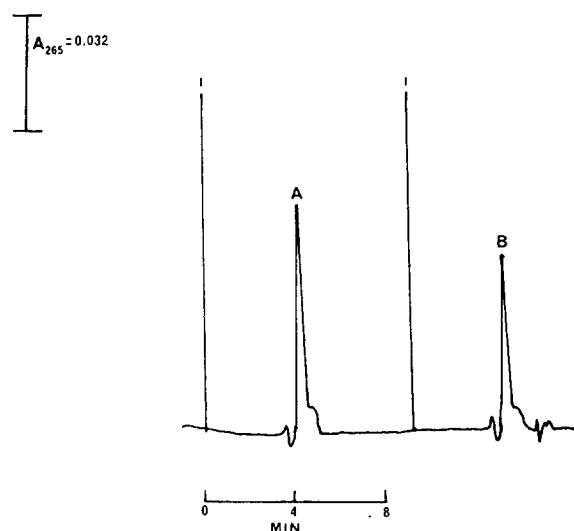


Fig.1. HPLC evidence for reaction of ascorbic acid with HOCl. HPLC was carried out on a pre-packed 4.6 mm \times 25 cm Anachem S5 ODS-2 column with an Anachem S5 ODS-2 guard column [18–20]. The mobile phase was 90% (v/v) 30 mM sodium citrate/27.7 mM sodium acetate buffer, pH 7.4, 10% (v/v) methanol at a flow rate of 1.0 ml $\cdot \text{min}^{-1}$, continuously sparged with helium. Ascorbic acid was detected by its absorbance at 265 nm. 27 μM ascorbic acid in 20 mM KH_2PO_4 -KOH buffer, pH 7.4, was incubated with 25 μM HOCl for 20 min at 37°C. Peak A, control incubation (no HOCl added); peak B, HOCl present. The ascorbic acid concentration decreased to 17 μM . If 50 μM HOCl was present, [ascorbic acid] decreased to 5 μM and 100 μM HOCl decreased [ascorbic acid] to 1.5 μM . No oxidation products of ascorbic acid were observed with the UV detector. i, injection spike.

If the α_1 -antiprotease is exposed to HOCl before adding elastase, its ability to inhibit is lost (column C, first row) and elastase activity is high. The HOCl concentration chosen is within the range of concentrations likely to be produced adjacent to myeloperoxidase in vivo [13,14,22]. Column C also shows the effect of including ascorbic acid in the reaction mixture with HOCl and α_1 -antiprotease. It seems that ascorbic acid, at physiologically relevant concentrations, is able to protect α_1 -antiprotease against inactivation by HOCl, so that it can still inhibit elastase. This protective effect of ascorbic acid is greatly enhanced by pre-incubating it with HOCl before adding the α_1 -antiprotease, suggesting that ascorbic acid is acting by scavenging HOCl (column D). On the other hand, ascorbic acid cannot restore the activity of α_1 -antiprotease after HOCl treatment (not shown).

In order to confirm a reaction of ascorbic acid with HOCl, HPLC analysis was used. Fig.1 shows that incubation of ascorbic acid with HOCl at pH 7.4 caused its disappearance.

4. DISCUSSION

The results in the present paper confirm previous suggestions that HOCl reacts with ascorbic acid [4,14]. More importantly, they show that this effect is biologically significant, in that the concentrations of ascorbic acid normally present in serum and synovial fluid [10,11,15,16] can offer protection to the important biological target α_1 -antiprotease against inactivation by HOCl produced by myeloperoxidase [12]. In serum, albumin is an effective HOCl scavenger [17], but albumin concentrations in synovial fluid are much lower than in serum [21] and so the protective effect of ascorbic acid against HOCl produced by myeloperoxidase may well be very significant at this site. The rapid depletion of ascorbic acid at sites of inflammation, as in rheumatoid arthritis, by reaction with O_2^- [4] and $\cdot OH$ [1,7], will thus be expected to lead to increased protease activity consequent upon α_1 -antiprotease inactivation, and an exacerbation of damage.

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REFERENCES

- [1] Anbar, M. and Neta, P. (1967) *Int. J. Appl. Radiat. Isot.* 18, 493–523.
- [2] Nishikimi, M. (1975) *Biochem. Biophys. Res. Commun.* 63, 463–468.
- [3] Arad, I.D., Forman, H.J. and Fisher, A.B. (1980) *J. Lab. Clin. Med.* 96, 673–681.
- [4] Hemilä, H., Roberts, P. and Wikström, M. (1984) *FEBS Lett.* 178, 25–30.
- [5] Halliwell, B. (1984) *Chloroplast Metabolism*, Clarendon Press, Oxford.
- [6] Winterbourn, C.C. (1979) *Biochem. J.* 182, 625–628.
- [7] Rowley, D.A. and Halliwell, B. (1983) *Clin. Sci.* 64, 649–653.
- [8] Czapski, G. and Goldstein, S. (1986) *Free Radical Res. Commun.* 1, 157–161.
- [9] Winterbourn, C.C. and Vissers, M.C.M. (1983) *Biochim. Biophys. Acta* 763, 175–179.
- [10] Blake, D.R., Hall, N.D., Treby, D.A., Halliwell, B. and Gutteridge, J.M.C. (1981) *Clin. Sci.* 61, 483–486.
- [11] Lunec, J. and Blake, D.R. (1985) *Free Radical Res. Commun.* 1, 31–39.
- [12] Weiss, S.J. (1986) *Acta Physiol. Scand. suppl.* 548, 9–37.
- [13] Albrich, J.M., McCarthy, C.A. and Hurst, J.K. (1981) *Proc. Natl. Acad. Sci. USA* 78, 210–214.
- [14] Winterbourn, C.C. (1985) *Biochim. Biophys. Acta* 840, 204–210.
- [15] Koch, P., Sidloi, M. and Tonks, D.B. (1980) *Clin. Biochem.* 13, 73–77.
- [16] Samyn, W. (1983) *Clin. Chim. Acta* 133, 111–117.
- [17] Wasil, M., Halliwell, B., Hutchison, D.C.S. and Baum, H. (1987) *Biochem. J.*, in press.
- [18] Grootveld, M., Halliwell, B. and Moorhouse, C.P. (1987) *Free Radical Res. Commun.*, in press.
- [19] Grootveld, M. and Halliwell, B. (1986) *Biochem. J.* 237, 499–504.
- [20] Grootveld, M. and Halliwell, B. (1986) *Free Radical Res. Commun.* 1, 243–250.
- [21] Gutteridge, J.M.C. (1986) *Biochim. Biophys. Acta* 869, 119–127.
- [22] Kalyanaraman, B. and Soehnle, P.G. (1985) *J. Clin. Invest.* 75, 1618–1622.