Effect of ascorbic acid on the production of singlet oxygen by purified human myeloperoxidase

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Received 1 May 1985

We have previously studied purified human myeloperoxidase-hydrogen peroxide-halide ion systems as models of possible singlet oxygen production by granulocytes. While myeloperoxidase could efficiently produce singlet oxygen, the yield of singlet oxygen at a physiological pH with Cl^- was very small due to enzyme inactivation. In that Bolscher et al. [(1984) Biochim. Biophys. Acta 784, 189–191] observed that micromolar concentrations of ascorbic acid prevented inactivation of myeloperoxidase and increased the production of hypochlorous acid, we examined whether ascorbic acid would augment singlet oxygen production by the myeloperoxidase-hydrogen peroxide-halide ion systems. Ascorbic acid, however, fails to increase the singlet oxygen yield, suggesting that it does not augment singlet oxygen production in the intact granulocyte by a myeloperoxidase-dependent mechanism.

Ascorbic acid *Myeloperoxidase* *Singlet oxygen*

1. INTRODUCTION

The biochemical oxidizing system consisting of myeloperoxidase, H_2O_2 and Cl^- has been extensively studied as a model for the production of microbicidal and cytotoxic oxidant species by the human neutrophil [1,2]. While recent reports have emphasized the importance of HOCl as a major mediator of this system [3–6], whether another highly reactive species, singlet oxygen ($^1O_2$) is also produced in significant quantities has been much debated [7–15]. In a recent study, we detected the characteristic 1268 nm $^1O_2$ emission band in the myeloperoxidase-H_2O_2-halide systems and quantitated the yield of $^1O_2$ under a variety of conditions [16]. At acid pH, the production of $^1O_2$ was stoichiometric with Br^- and modestly efficient with Cl^-, but at the more alkaline pH characteristic of the phagocytic vacuole, the production of $^1O_2$ was very small [16–18]. Enzyme inactivation was a major factor limiting the yield of $^1O_2$. The recent study of Bolscher et al. [19] caused us to question the validity of the purified myeloperoxidase model. They demonstrated that the presence of micromolar concentrations of ascorbic acid protected the myeloperoxidase from inactivation. This raised the optimal pH for HOCl formation and produced a 3-fold increase in the HOCl yield [19]. Since the concentration of ascorbic acid in the human granulocyte is 150 μM [20,21], we undertook a study of the effect of ascorbic acid on the $^1O_2$ yield of the myeloperoxidase-H_2O_2-halide systems.

2. MATERIALS AND METHODS

Details of the construction of the infrared chemiluminescence spectrometer, method of quantitation of $^1O_2$ production using the H_2O_2 + HOCl reaction, purification of human myeloperoxidase and source of the reagents used have been reported previously [16,22,23]. The sample of myeloperoxidase used for this study had an $A_{430}/A_{280} = 0.7$ and an activity of 98 units/mg defined using

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00145793/85/$3.30

4-aminoantipyrine as a hydrogen donor [24]. Ascorbic acid was obtained from Sigma (St. Louis, MO).

3. RESULTS AND DISCUSSION

Fig.1 shows the effect of the addition of various amounts of ascorbic acid to the myeloperoxidase-H_2O_2-halide systems under conditions where the yield of $^1O_2$ was low due to enzyme inactivation [16]. No significant increase in $^1O_2$ production was observed at any concentration. High concentrations of ascorbic acid decreased the yield of $^1O_2$. Fig.2 demonstrates the effect of ascorbic acid on the kinetics of $^1O_2$ production at p^2H 4 with Cl^-. Here the reaction rate is slow, but enzyme inactivation is less important. Increasing concentrations of ascorbic acid caused a progressive delay in the onset of the chemiluminescence.

We have previously used these highly purified myeloperoxidase systems as models of possible $^1O_2$ production by the intact granulocyte [16]. We reasoned that the purified myeloperoxidase system would set an upper limit to any $^1O_2$ production by granulocytes via a myeloperoxidase-dependent mechanism, since the large concentration of biomolecules present in the granulocyte would compete with the H_2O_2 for any HOCl produced, thus decreasing the amount of $^1O_2$ formed [3,4,16,25]. An alternative hypothesis that we did not consider was that the presence of ascorbic acid or other reducing substances would protect the myeloperoxidase from inactivation and increase the $^1O_2$ yield. The data presented here demonstrate that this is not the case. Consistent with this study is the fact that we were unable to detect any 1268 nm emission from human granulocytes stimulated with phorbol myristate acetate in Cl^--containing buffers [26]. Unfortunately, in experiments with intact granulocytes, the anticipated luminescence is near the limit of sensitivity of the current spectrometer, so that small, but significant amounts of $^1O_2$ may go undetected.

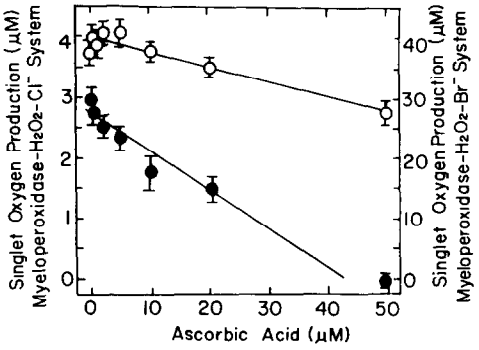

Fig.1. Effect of ascorbic acid on the yield of singlet oxygen in the myeloperoxidase-H_2O_2-halide systems. (●) p^2H 6, 100 mM sodium phosphate, 200 nM myeloperoxidase, 1 mM H_2O_2, 100 mM NaCl, deuterium oxide solvent; (○) p^2H 7, 100 mM sodium phosphate, 270 nM myeloperoxidase, 1 mM H_2O_2, 100 mM NaBr, deuterium oxide solvent.

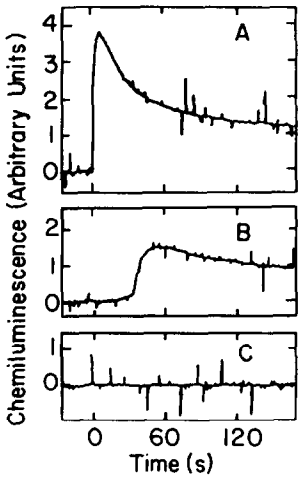

Fig.2. Effect of ascorbic acid on the kinetics of singlet oxygen production in the myeloperoxidase-H_2O_2-Cl^- system. Conditions: p^2H 4, 100 mM sodium acetate, 130 nM myeloperoxidase, 100 mM NaCl, deuterium oxide solvent. (A) No ascorbic acid; (B) 300 μM ascorbic acid; (C) 1 mM ascorbic acid.

Our myeloperoxidase system differs in an important way from that described by Bolscher et al. [19]. The monochlorodimedone in their system prevented the accumulation of significant concentrations of HOCl. In our Cl^- system, moderate concentrations of HOCl are required for the formation of $^1O_2$, since the H_2O_2 + HOCl reaction is relatively slow. Under these conditions HOCl may irreversibly inactivate myeloperoxidase. Naskalski [27] has shown that 3 molecules of HOCl are sufficient to destroy the heme group in myeloperoxidase and that this process cannot be reversed by ascorbic acid. This contrasts with the study of Bolscher et al. [19] where the major mechanism of

inactivation appeared to be the reversible formation of Compound II. In our study, the most likely explanation for the decreased $^1O_2$ yield caused by ascorbic acid is a direct reaction between HOCl and ascorbic acid. The kinetic data presented in fig.2 support this, since all of the ascorbic acid must be oxidized before any $^1O_2$ is produced. The reaction of HOCl with ascorbic acid may be fast enough to prevent the formation of $^1O_2$, but not fast enough to prevent the inactivation of myeloperoxidase. Ascorbic acid is a well known quencher of $^1O_2$, but in this study, the concentrations of ascorbic acid were too low to cause significant quenching. A concentration of 7 mM is required to cause 50% quenching in deuterium oxide buffers [28].

ACKNOWLEDGEMENTS

We wish to thank William Wardman for technical assistance in performing experiments and Tena Beavers for assistance in preparation of the manuscript. This work is supported by grants GM-32974 and AI-20064 from the National Institutes of Health, the Veterans Administration Research Service and the Neutrophil Fund, Inc. J.W. is the recipient of an Arthritis Foundation Research Fellowship.

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