ASCORBIC ACID AS A SCAVENGER OF SINGLET OXYGEN

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1. Introduction

The role of the reactive forms of oxygen in physiologic and pathologic processes is a topic under intense investigation at the present time. Similarly, the role of biological anti-oxidants such as ascorbic acid and tocopherol as inhibitors of and therapy for pathologic processes is a subject of considerable experimental and clinical interest. The reactive forms of oxygen include $O_2^{\cdot-}$, $OH^{\cdot}$, H_2O_2, $^1O_2$ and metal ion–oxygen complexes. Nishikimi [1] has shown that ascorbate can scavenge $O_2^{\cdot-}$ with a second-order rate constant of 2.7×10^5 $M^{-1}.s^{-1}$. Bielski et al. [2] have demonstrated ascorbate to be an effective scavenger for $OH^{\cdot}$ at neutral pH.

Singh and Vadasz [3] demonstrated that ascorbate inhibited the methylene blue-catalyzed photoinactivation of *Escherichia coli* ribosomes by 60% and suggested that the inhibition was mediated via the scavenging of $^1O_2$ by ascorbate. This communication presents further experimental evidence indicating that ascorbate is a $^1O_2$ scavenger.

2. Materials and methods

Singlet oxygen was generated photochemically using ultraviolet light and hematoporphyrin as a sensitizer [4–6]. The apparatus and light source have been described [6]. A typical reaction mixture contained 8 μM hematoporphyrin, 10 mM potassium phosphate (pD 7.5), 0.1 mM EDTA and 0.1 mM ascorbate. Deuterium oxide (99.8%) was used to make solutions. The oxidation of ascorbate was monitored by the decrease in A_{265} [1].

3. Results and discussion

Singlet oxygen has a mean lifetime of 20 μs in D_2O as compared to 2 μs in H_2O [7–9]. As can be seen in table 1 the reaction in D_2O shows a 4.7-fold potentiation over that in H_2O. The effects of additions of methionine, a well known singlet oxygen scavenger, and of azide, a well-known singlet oxygen quencher, were also examined. Methionine reacts with singlet oxygen with a rate constant of 3×10^7 $M^{-1}.s^{-1}$ [10]. Table 1 shows that 5.0 mM methionine causes a 49% inhibition of the rate of ascorbate photooxidation. The rate constant for the quenching of $^1O_2$ by azide ion is 2.2×10^8 $M^{-1}.s^{-1}$ [11]. Table 1 shows that 0.25 mM azide results in a 60% inhibition of ascorbate photooxidation. This suggests that the reactivity of ascorbate toward singlet oxygen is approximately of the same order of magnitude as azide.

Photosensitized oxidations can proceed by either a type I or a type II mechanism. A type I mechanism

Table 1
Effect of singlet oxygen reactive compounds on the hematoporphyrin-catalyzed photooxidation of ascorbate

Addition	ΔA_{265} (% control)
None	(100)
H_2O in place of D_2O	22
Methionine 2.5 mM	68
Methionine 5.0 mM	51
Azide 0.10 mM	75
Azide 0.25 mM	40

The experimental conditions are described under section 2. The control values ranged from 0.28–0.36 *A* units. Duration of irradiation is 1 min at 400 μW/cm²

involves direct interaction between the excited sensitizer and the substrate. A type II mechanism involves $^1O_2$ as the direct interactant with the substrate. Hematoporphyrin has been shown to produce $^1O_2$ [4–6]. Therefore, in the event that the photosensitized oxidation of ascorbate proceeds entirely via a type I mechanism (and none of the oxidation by type II), an increase of oxygen concentration to compete for the excited state of the sensitizer would be expected to decrease the rate of ascorbate oxidation. When the oxygen concentration was increased 5-fold by saturating the medium with prepurified oxygen (Matheson) there was no detectable change in the rate of ascorbate oxidation as compared with that in the air-saturated sample (data not shown). This suggests that if a type I process is operational here, it makes a minor contribution; the major mechanism being that mediated by $^1O_2$.

An attempt was also made to exclude $O_2^{\cdot-}$ as the mediator of the reaction. Addition of superoxide dismutase (15 μg/ml) in the photooxidation medium caused only a 10% decrease in the rate of ascorbate oxidation, suggesting that $O_2^{\cdot-}$ is not the major photooxidative species in this system.

Taken collectively these results suggest that in addition to its ability to scavenge $O_2^{\cdot-}$ and $OH^{\cdot}$, ascorbate can also scavenge $^1O_2$.

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