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Measurement and characterization of singlet oxygen production in copper ion-catalyzed aerobic oxidation of ascorbic acid

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Abstract

Production of singlet molecular oxygen ($^1O_2$) in the aerobic oxidation of ascorbic acid catalyzed by copper ion was measured and characterized using [4′-(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)tetrakis(acetate)-Eu^{3+} (ATTA-Eu^{3+}) as a highly sensitive and selective time-resolved luminescence probe for $^1O_2$. The $^1O_2$ produced in the reaction was further characterized and confirmed by (i) chemical trapping of $^1O_2$ with 9,10-diphenylanthracene (DPA), the corresponding endoperoxide was detected by HPLC and (ii) spin trapping of $^1O_2$ with 2,2,6,6-tetramethyl-4-piperidinol (TMP-OH), the corresponding free radical of TMP-OH oxide (TMPO$^\bullet$) was detected by electron spin resonance (ESR) spectroscopy. The effects of deuterium oxide, sodium azide and histidine on the $^1O_2$ signal were investigated. The mechanism investigation of $^1O_2$ production implied that the ascorbic acid–Cu(I) complex formed in the reaction could be an important intermediate for the $^1O_2$ production. The reaction of ascorbic acid with copper ion monitored by ^{1}H NMR and absorption spectroscopy demonstrated the formation of a copper ion–ascorbic acid complex. Except for Cu^{2+} and Cu^+–ascorbic acid systems, no detectable $^1O_2$ was produced in other transition metal cation–ascorbic acid systems in the studied range.

Keywords: Ascorbic acid; Aerobic oxidation; Europium; Singlet oxygen production; Time-resolved luminescence

1. Introduction

Ascorbic acid (or Vitamin C) is ubiquitous to living systems, and a wide variety of its biological functions have been proposed. Experimental and epidemiological studies have demonstrated that ascorbic acid has anticarcinogenic and chemopreventive actions [1,2] by scavenging physiologically relevant reactive oxygen species and reactive nitrogen species. As an effective water-soluble antioxidant in plasma, ascorbic acid can prevent lipid peroxidation induced by peroxyl radicals or the gas-phase of cigarette smoke, and possibly protect against cardiovascular disease [3,4]. This has led to the proposal that dietary supplementation with ascorbic acid may be useful in disease prevention. Paradoxically, ascorbic acid is also known to act as a pro-oxidant in vitro, the mechanisms underlying these actions are still unclear. Ascorbic acid can assist metal ion-induced oxidative modifications of lipid, protein and DNA [5–7]. Rosenthal and Benhur have shown that the photohaemolysis rate of human red blood cells sensitized by chloroaluminium phthalocyanine sulphonate can be increased by ascorbic acid [8]. A mechanism that ascorbic acid induces the decomposition of lipid hydroperoxides to genotoxic bifunctional eletrophiles in vitro has been provided [9]. In the presence of the redox active metal ion, ascorbic acid may contribute to oxidative damage of biomolecules by ascorbic acid-induced Fenton reaction [10], or by H_2O_2 and $O_2^{\bullet-}$ produced in the oxidation of ascorbic acid by molecular oxygen [11]. However, until now there is still no unequivocal evidence to demonstrate the $^1O_2$ production in the metal ion-mediated aerobic oxidation of ascorbic acid.

In some biological systems, $^1O_2$ is thought to be an important toxic species in vivo since it can oxidize various kinds of biological molecules such as proteins, DNA and lipids [12–15]. Singlet oxygen has been proposed to be involved in the cell signaling cascade and in the induction of gene expression [16,17]. Furthermore, it was suggested that $^1O_2$ should be involved in changes in the mitochondrial membrane pore transition [18]

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and be a key to the bactericidal response of certain antibodies [19,20]. Regarding photoinduced processes, $^1O_2$ is believed to be involved in the photosensitivity of patients with erythropoietic protoporphyria and drug phototoxicity [21,22], and its formation has been extensively employed therapeutically [23]. In addition, there is overwhelming evidence that excited state species produced by biochemical reactions in cells and tissues exposed to oxidative stress may subsequently react with ground-state oxygen to yield $^1O_2$ as in photosensitization [24].

Time-resolved fluorometry combined with the use of lanthanide complex-based luminescence probes has provided an excellent way for developing highly sensitive bioaffinity assays [25,26]. The most important advantage of this technique is that the method can effectively eliminate the short-lived background noise from the biological samples and optical components. We have recently demonstrated that an europium(III) complex, [4′-(9-anthryl)-2,2′:6′,2″-terpyridine-6,6″-diyl]bis(methylenenitrilo)tetrakis (acetate)-Eu^{3+} (ATTA-Eu^{3+}), can be used as a highly sensitive and selective time-resolved luminescence probe for $^1O_2$ [27]. In the present work, the $^1O_2$ production in the aerobic oxidation of ascorbic acid catalyzed by copper ion was investigated by using ATTA-Eu^{3+} as a probe. To confirm the $^1O_2$ production in the system, the reaction was further characterized by using 9,10-diphenylanthracene (DPA) and 2,2,6,6-tetramethyl-4-piperidinol (TMP-OH) as chemical trapping and electron spin resonance (ESR) probes for $^1O_2$, the corresponding products of $^1O_2$ reacted with DPA and TMP-OH were detected by HPLC [28] and ESR spectroscopy [29], respectively. Effects of various reaction conditions on the yield of $^1O_2$ and the $^1O_2$ production mechanism in the system were also investigated.

2. Experimental

2.1. Chemicals and materials

Ascorbic acid, 2,2,6,6-tetramethyl-4-piperidinol and 9,10-diphenylanthracene were purchased from Acros Organics. Deuterium oxide (99.9%) was obtained from Cambridge Isotope Laboratories, Inc., dehydroascorbic acid (DHA) was obtained from Aldrich. Superoxide dismutase (SOD) from bovine erythrocytes (specific activity, 4520 units/mg), and catalase from bovine liver (specific activity, 2860 units/mg) were obtained from Sigma. Prior to use, hydrogen peroxide was diluted immediately from a stabilized 30% solution, and was assayed by using its molar absorption coefficient of $43.6\,M^{-1}\,cm^{-1}$ at 240 nm [30]. The ATTA–Eu^{3+} complex was synthesized by using a previous method [27]. The ATTA-Eu^{3+} endoperoxide (EP-ATTA-Eu^{3+}) and 9,10-diphenylanthracene endoperoxide ($DPAO_2$) were synthesized according to the literature methods [27,31], respectively. Unless otherwise stated, all chemical materials were purchased from commercial sources and used without further purification.

2.2. Time-resolved luminescence assay

All reactions were carried out in 0.05 M Tris–HCl buffer of pH 7.4 with 100 nM ATTA-Eu^{3+}, 1.0 mM ascorbic acid and 10 μM $CuSO_4$ at room temperature under air for 1 h. The time-resolved luminescence measurement was carried out on a Perkin-Elmer Victor 1420 Multilabel Counter with the measurement conditions of excitation wavelength, 340 nm; emission wavelength, 615 nm; delay time, 0.2 ms; window time (counting time), 0.4 ms; cycling time, 1.0 ms.

2.3. ESR assay

The H_2O-based or D_2O-based solution (10 ml) containing 1.0 mM ascorbic acid, 20 μM $CuSO_4$ and 50 mM TMP-OH were stirred for 5 h at room temperature under oxygen. To the solution were added ascorbic acid (2 mg) and 20 μl $CuSO_4$ (10 mM) at intervals of 1 h during the reaction. After the reaction, the solution was injected into the quartz capillary for ESR analysis. The ESR spectra were recorded at room temperature on a JES-FE1XG X-band spectrometer (JEOL, Japan) with 100-kHz field modulation and the settings of central field, 3360 G; sweep width, 250 G; microwave power, 5 mV; response 0.1 s.

2.4. HPLC assay

A mixture of 5 ml H_2O (or D_2O), 5 ml tetrahydrofuran, 1.8 mg ascorbic acid, 10 μl $CuSO_4$ (50 mM) and 13.2 mg DPA was stirred for 6 h at 37 °C under oxygen. To the solution were added ascorbic acid (2 mg) and 10 μl $CuSO_4$ (50 mM) at intervals of 1 h during the reaction. The solution was extracted with $CHCl_3$ (2×20 ml). The organic phase was dried with Na_2SO_4 and evaporated. The residue was dissolved in acetone, and then used for HPLC analysis. The HPLC analysis was carried out on an HPLC instrument (Micro-Tech Scientific Inc.) equipped with a 150 mm × 0.32 mm C_{18} reverse-phase column and a linear UV-200 detector, using the increasing linear gradient of acetonitrile/H_2O from 60 to 95% with flow-rate of 3.0 ml/min. The elution was monitored at 210 nm.

2.5. ^1H NMR assay

Reaction of 50 mM $CuSO_4$ with 50 mM ascorbic acid (or DHA) was carried out in D_2O at room temperature. The ^1H NMR spectra were measured on a Bruker DRX 400 spectrometer (400 MHz).

2.6. Absorption spectrum assay

Reaction of 0.1 mM ascorbic acid with 0.1 mM $CuSO_4$ (or CuCl) was carried out in 0.05 M NH_4Cl buffer of pH 6.5 at room temperature. The absorption spectra were recorded on a Perkin-Elmer Lambda 35 UV–vis spectrometer.

3. Results and discussion

3.1. Singlet oxygen production in copper ion-catalyzed aerobic oxidation of ascorbic acid

Europium(III) complex, ATTA-Eu^{3+}, is a highly sensitive and selective time-resolved luminescence probe for $^1O_2$ [27].