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Chemiluminescence evaluation of oxidative damage to biomolecules induced by singlet oxygen and the protective effects of antioxidants

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Abstract

A chemiluminescence (CL) method was developed for the evaluation of oxidative damage to biomolecules induced by singlet oxygen ($^1O_2$) and for the evaluation of the protective effects of antioxidants. The $^1O_2$ was generated from the reaction of $H_2O_2+OCl^-$. Results showed that the CL signal from the reaction of $H_2O_2+OCl^-$ was weak, however, it was enhanced dose-dependently with the addition of DNA and unsaturated fatty acid, respectively. Spectra analysis indicated that the enhanced CL could be ascribed to the decay of triplet-excited carbonyl compounds, which were generated from the reaction of $^1O_2$ plus the biomolecules. On the other hand, the enhanced CL produced in the above systems could be effectively inhibited by lycopene, β-carotene, VC, and VE, but could not be inhibited by mannitol, SOD, and NaN_3. The mechanism therein was discussed.

Keywords: Antioxidant; Singlet oxygen; Oxidative damage; Chemiluminescence

1. Introduction

Singlet oxygen ($^1O_2$) is one of reactive oxygen species generated in biological systems [1]. A large number of literatures [2–6] indicate that it can oxidatively damage biomolecules such as DNA, proteins, lipids, etc., initiating cancer and cardiovascular diseases. The methods used in those studies are usually based on the measurement of the final oxidative products of biomolecules by means of high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), ultraviolet spectrometry (UV), and biochemical or chemical analysis, which make it is easy to reveal the types and amounts of the damaged substrates, but is hard to trace the whole reaction processes online due to the limitation in sampling, preparation of sample, etc.

It has been reported that chemiluminescence (CL) can be produced in the process of oxidative damage of biomolecules [7], which seems to be one of the earliest responses, increasing before any other changes may be detected, thus making it a useful parameter for tracing the process of oxidative damage, especially for tracing the early stage. Some authors have taken trials in establishing CL method for the evaluation of OH^--induced oxidative damage to DNA, which not only opens a new way of understanding the mechanism involved in the oxidative damage, but also presents a simple, quick, and effective method for the screening of antioxidants for prevention [8]. However, few studies have been involved in the CL from $^1O_2$-induced oxidative damage to biomolecules, to my knowledge.

The purpose of this paper is to develop a CL method for the evaluation of the oxidative damage of biomolecules induced by $^1O_2$ and for the evaluation of the protective effects of antioxidants. In our previous work, we established the generation system of $^1O_2$ based on the reaction between peroxide hydrogen and sodium hypochlorite ($H_2O_2+OCl^-$) [9]. In this study, the generation system is used as $^1O_2$ source. Calf thymus DNA and linolenic acid (one of the

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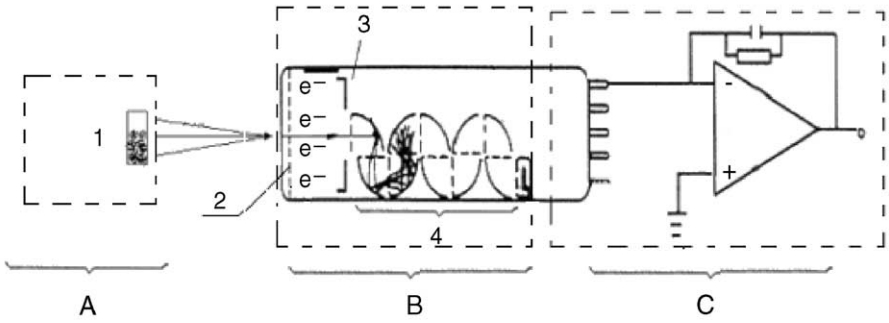

Fig. 1. Structure of chemiluminescence detector. 1—sample cell; 2—light—electron conversion surface; 3—focusing electrode; 4—electron multiplier tube. A—Sample support; B—photon counting photomultiplier; C—data processor.

main unsaturated fatty acids in lipid membranes) are used as model compounds for biomolecules.

2. Materials and methods

2.1. Chemicals

Lycopene, β-carotene, sodium azide (NaN_3), mannitol, SOD, calf thymus DNA, and linolenic acid (UFA) were purchased from Sigma. Co. (St. Louis, MO). Vitamin C (VC), Vitamin E (VE), NaOCl, and H_2O_2 were purchased from Shanghai Bio-chemical Agent Shop (Shanghai, China).

2.2. CL detector

CL was detected on a SH-G type chemiluminescence detector (CD), as shown in Fig. 1 (Shanghai Measurement Equipment Factory, Shanghai, China). The CD was similar to that described by Ma et al. [8], with some modification, which was composed of three parts: a sample support A, in which a sample cell (glass tube, diameter=10 mm, height=20 mm) could be placed; a photon counting photo-

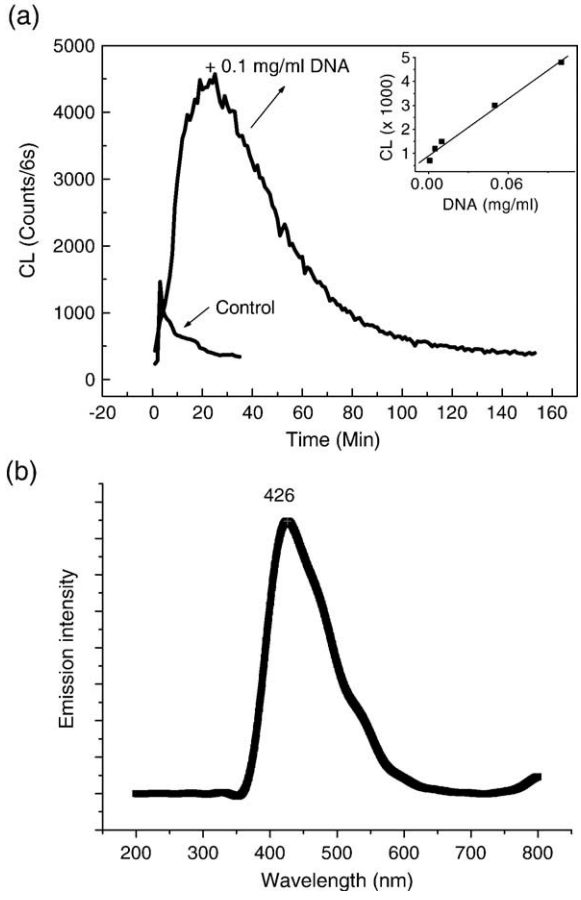

Fig. 2. Time-dependent CL curves (a) and spectrum (b) of DNA oxidized by $^1O_2$.

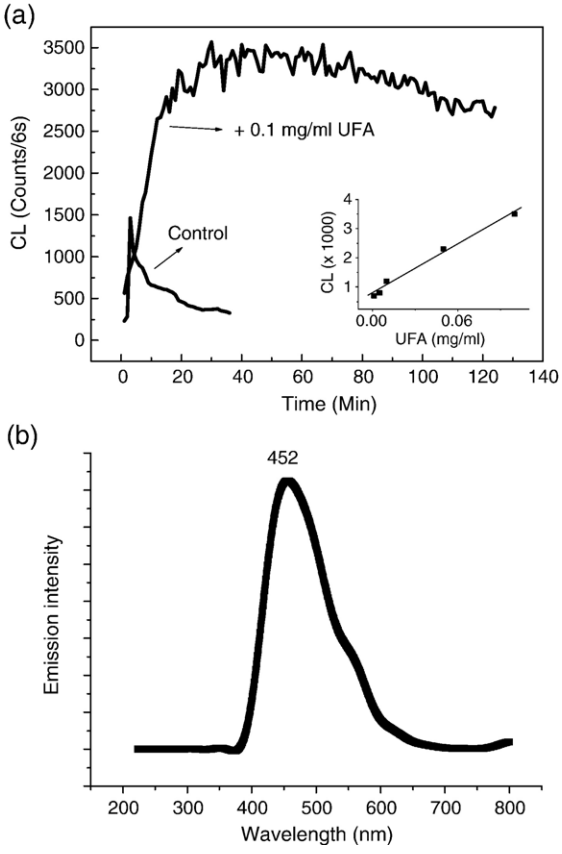

Fig. 3. Time-dependent CL curves (a) and spectrum (b) of UFA oxidized by $^1O_2$.