Specific Chemiluminescence from Singlet Oxygen Generated by the Reaction of Acetonitrile and Hydrogen Peroxide in the Presence of Alkali Halide

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We developed a detection apparatus, equipped with two photomultiplier tubes, for chemiluminescence from singlet oxygen: the dimol light emission (ca. 634 nm) and the monomol light emission (ca. 1270 nm). Singlet oxygen was generated by reactions between sodium hypochlorite and hydrogen peroxide and between acetonitrile and hydrogen peroxide, and the dimol and monomol chemiluminescence emission were simultaneously observed using the apparatus. It was found that the chemiluminescence of the monomol emission in the acetonitrile system was enhanced in the presence of alkali halides and lasted more than 1.5 h, while the dimol emission in the acetonitrile system and the dimol and monomol emission in the hypochlorite system were not affected by the presence of alkali halide. The effects of antioxidants and nucleosides on monomol chemiluminescence in the acetonitrile system were examined. The specific chemiluminescence of the monomol emission of singlet oxygen in the acetonitrile system in the presence of alkali halides and the initial data concerning antioxidants and nucleosides will be useful in the design of new analytical systems or in elucidating the mechanism of chemiluminescence emission from singlet oxygen.

Active oxygen has attracted a great deal of attention, not only as a species in chemical reactions but also in the area of medical science from the point of view of disease factors, health maintenance, and aging.[1,2] Active oxygen species include singlet oxygen, hydroxyl radicals, and superoxide radical anions. It is known that singlet oxygen is generated by the reaction of sodium hypochlorite and hydrogen peroxide and also by that of acetonitrile and hydrogen peroxide. Singlet oxygen possesses unique chemiluminescence (CL) properties, emitting dimol (ca. 634 nm) and monomol (ca. 1270 nm) CL.[3,4]

In this study, we developed a detection apparatus for CL from singlet oxygen (dimol and monomol emission). We simultaneously observed CL around 634 and 1270 nm from singlet oxygen generated by the sodium hypochlorite/hydrogen peroxide and acetonitrile/hydrogen peroxide reactions. It was found that the CL of the monomol emission in the acetonitrile system was enhanced in the presence of alkali halides such as sodium chloride and potassium chloride, lasting more than 1.5 h. This was an unexpected phenomenon, because even commercial CL analysis reagents, such as luminol, lucigenin, TDPO (bis[2-(3,6,9-trioxadecanyloxycarbonyl)-4-nitrophenyl] oxalate), and MPEC (2-methyl-6-*p*-methoxyphenylethynylimidazopyrazinone), do not show CL lasting more than several tens of minutes with sufficient intensity to allow analytical procedures.

Water was purified using an Elix UV 3 system (Millipore). All reagents used were commercially available and were of special analytical grade. Sodium hypochlorite, acetonitrile, hydrogen peroxide solution (30 wt %), and sodium azide were purchased from Nacalai Tesque. 2′-Deoxyguanosine (dGuo), 2′-thymidine (dThd), 2′-deoxyadenosine (dAdo), and 2′-deoxycytidine (dCyd) were from Wako Pure Chemical Industries.

The experiments using the CL detection apparatus equipped with the two kinds of photomultiplier tube (PMT) were carried out as follows: For the hypochlorite system, 10 mM hydrogen peroxide solution (100 mM carbonate buffer, pH 10.8) (1 mL) was added to 10 mM sodium hypochlorite solution (100 mM carbonate buffer, pH 10.8) (5 mL) in the reaction cell through a PTFE tube. Similarly, for the acetonitrile system, 5 M hydrogen peroxide solution (100 mM carbonate buffer, pH 10.8) (1 mL) was added to acetonitrile solution (acetonitrile/100 mM carbonate buffer, pH 10.8, 1/1 v/v) (5 mL) in the cell. To examine the effects of alkali halides, these were placed in the reaction cell in advance. Dimol CL (ca. 634 nm) was detected using a PMT (R1924A, Hamamatsu; sensitive region, 300–650 nm) and a cut filter (52527-E, Edmund) (cut field <600 nm). Monomol CL (ca. 1270 nm) was detected using a PMT (H9170-45, Hamamatsu; sensitive region, 950–1400 nm), which was cooled to −20 °C by thermoelectric refrigeration under vacuum, and a band path filter (1260-11-45, Intor) (1260 nm ± 11.8 nm). For each reaction solution, dimol and monomol CL from singlet oxygen were simultaneously detected using the two PMTs. These were treated with an integrator (Chromatopac C-R8A, Shimadzu Co.).

First, we observed the CL profiles of both emission in the absence of alkali halides. There was a considerable difference in the CL profiles observed for the hypochlorite and acetonitrile systems. In the hypochlorite system,[5] the CL of both emission appeared quickly after injection of the hydrogen peroxide solution and disappeared within several seconds, while in the acetonitrile system, both CL emission gradually increased after injection of the hydrogen peroxide solution and lasted for several minutes. The half-life of singlet oxygen is short, of microsecond order, although this is dependent on the solvent[6] (e.g., 3.3 μs in water and 68.0 μs in heavy water). Therefore, the difference in the CL profiles must be based on the difference in the reaction rates of the hypochlorite and acetonitrile systems.

Next, we examined the effects of alkali halides, such as sodium chloride and potassium chloride, on the CL profiles from singlet oxygen. Figure 1 shows the CL profiles of the monomol emission in the acetonitrile system in the absence and presence of potassium chloride. Almost the same profile was observed for sodium chloride (data not shown). It was found that the monomol CL emission in the acetonitrile system was enhanced by the presence of alkali halides, with CL lasting for ca. 1.5 h. To confirm the specific CL emission, we closed (17 min after the

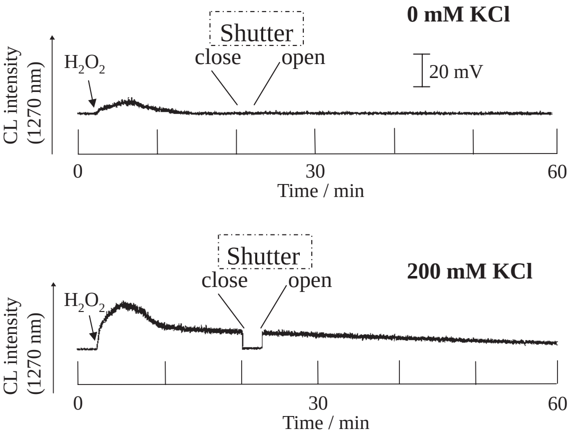

Figure 1. CL profiles of the monomol emission of singlet oxygen generated in the reaction between acetonitrile and hydrogen peroxide. Conditions: 5 M hydrogen peroxide in 100 mM carbonate buffer (pH 10.8) (1 mL) was added to acetonitrile/100 mM carbonate buffer (pH 10.8, 1/1 v/v) (5 mL) in the absence (top) and presence (bottom) of potassium chloride (200 mM).

injection of hydrogen peroxide) and opened the shutter in front of the PMT; as shown in the figures, addition of the salt resulted in greater and longer-lasting CL. Interestingly, the salts had no effect on the CL of the dimol emission in the acetonitrile system or the dimol or monomol emission in the hypochlorite system. The reason for this is not yet clear; the presence of an alkali halide in the acetonitrile/carbonate buffer (water) mixture solution may have considerably altered the reaction rate and/or the CL quantum yield of the monomol singlet oxygen emission.

Sodium azide, nitro blue tetrazolium, and 2-propanol are well-known antioxidants for active oxygen: sodium azide acts on singlet oxygen, nitro blue tetrazolium on superoxide radical anions, and 2-propanol on hydroxyl radicals. In addition, these reagents are known to be unreactive toward hydrogen peroxide. We examined the effect of these antioxidants on the monomol CL profile in the acetonitrile system in the presence of potassium chloride. Each antioxidant (10 mM, 0.5 mL) was added to the reaction cell 17 min after the injection of hydrogen peroxide, at which time the effects of the additives (CL quenching) could easily be observed against the stable CL, similarly to the shutter operation in Figure 1. The addition of sodium azide resulted in sudden quenching of the CL emission to about zero intensity, followed by a gradual revival to its former intensity within about 10 min. The addition of nitro blue tetrazolium and 2-propanol resulted in no change in the CL profiles compared to blank injections of buffer solution. These results support the proposition that the specific monomol CL emission is from singlet oxygen generated from the reaction between acetonitrile and hydrogen peroxide. When we tentatively examined the use of various concentrations of sodium azide (5×10^{-3}–5×10^{-2} M) in similar experiments, it was confirmed that there was a good linear relationship between the concentration of sodium azide and the quenching peak area.

Ravanat et al. examined the reactivity of various nucleosides (dGuo, dThd, dAdo, and dCyd) with singlet oxygen by analyzing the reaction products by mass spectrometry, but without distinguishing dimol and monomol;[7] they concluded that the order of reactivity was dGuo $\gg$ dAdo, dThd, dCyd. We examined these four nucleosides (20 mM) against monomol CL in the acetonitrile system with the addition 17 min after the injection of hydrogen peroxide. 2′-Deoxyguanosine (dGuo) showed a clear quenching effect, while the other nucleosides showed little quenching; based on an estimated quenching area for dGuo of 100, the areas of the quenching areas of the other nucleosides were less than 3.

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