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Effect of DABCO (1,4-diazabicyclo[2,2,2]-octane) on singlet oxygen monomol (1270 nm) and dimol (634 and 703 nm) emission

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Monomol (1270 nm) and dimol (634 and 703 nm) emissions of singlet oxygen arising from the H_2O_2–OCl^- reaction are compared by using a germanium detector and a red-sensitive photomultiplier, respectively. The detection limits in both cases are compared and the possibility of their application to biological systems in order to detect singlet molecular oxygen is discussed. 1,4-Diazabicyclo[2,2,2]-octane (DABCO) enhances both dimol and monomol emission of singlet oxygen from the above reaction; however, the tentative ratio 'monomol/dimol emission' is lowered in the presence of the tertiary amine.

Singlet oxygen *Monomol emission* *Dimol emission* *DABCO*

1. INTRODUCTION

Different methods are used to put in evidence the occurrence of singlet oxygen in biological systems. The most widespread include [1]: (i) the use of quenchers and traps; (ii) the measurement of low-level chemiluminescence at specific wavelengths; (iii) the increase of singlet oxygen life time in D_2O. The combination of two of these methods, identification of the red band chemiluminescence of singlet oxygen (dimol emission) and the effect of quenchers on it, is thought to produce reliable evidence for the occurrence of singlet oxygen in certain stages of biological oxidations. Singlet oxygen dimol emission takes place at 634 and 703 nm [2,3] (with a minimum at 668 nm) as a consequence of a simultaneous transition of singlet oxygen pairs in the $^1\Delta g$ state. This was stated as a criterion for the identification of singlet oxygen, excluding broad bands of red chemiluminescence [4,5], and was further applied to biological reactions such as the oxidation of hydroperoxides by cytochrome *P*-450 [6] and of arachidonate by prostaglandin-endoperoxide synthase [7]. The observation of monomol emission of singlet oxygen [8], i.e. emission at 1268 and 1407 nm, is often demanded and sometimes claimed as the 'unique and reliable evidence' for the occurrence of singlet oxygen in biological reactions.

1,4-Diazabicyclo[2,2,2]-octane (DABCO) has been described as a quencher of singlet oxygen due to its effect in preventing or retarding the oxidation of known reactive acceptors of singlet oxygen along with an inhibition of the 634 nm band in the gas phase [8,10]. However, authors in [4,5] described a stimulation of singlet oxygen dimol emission in aqueous solution by DABCO; this enhancement based on an increased singlet oxygen life time was thought to be accompanied by a change in the relationship between monomol emission and dimol emission. However, the mechanism of deactivation of singlet oxygen by DABCO and its enhancement of dimol emission in aqueous solutions remains to be elucidated.

We try to assess the feasibility of detecting monomol emission in biological systems and the

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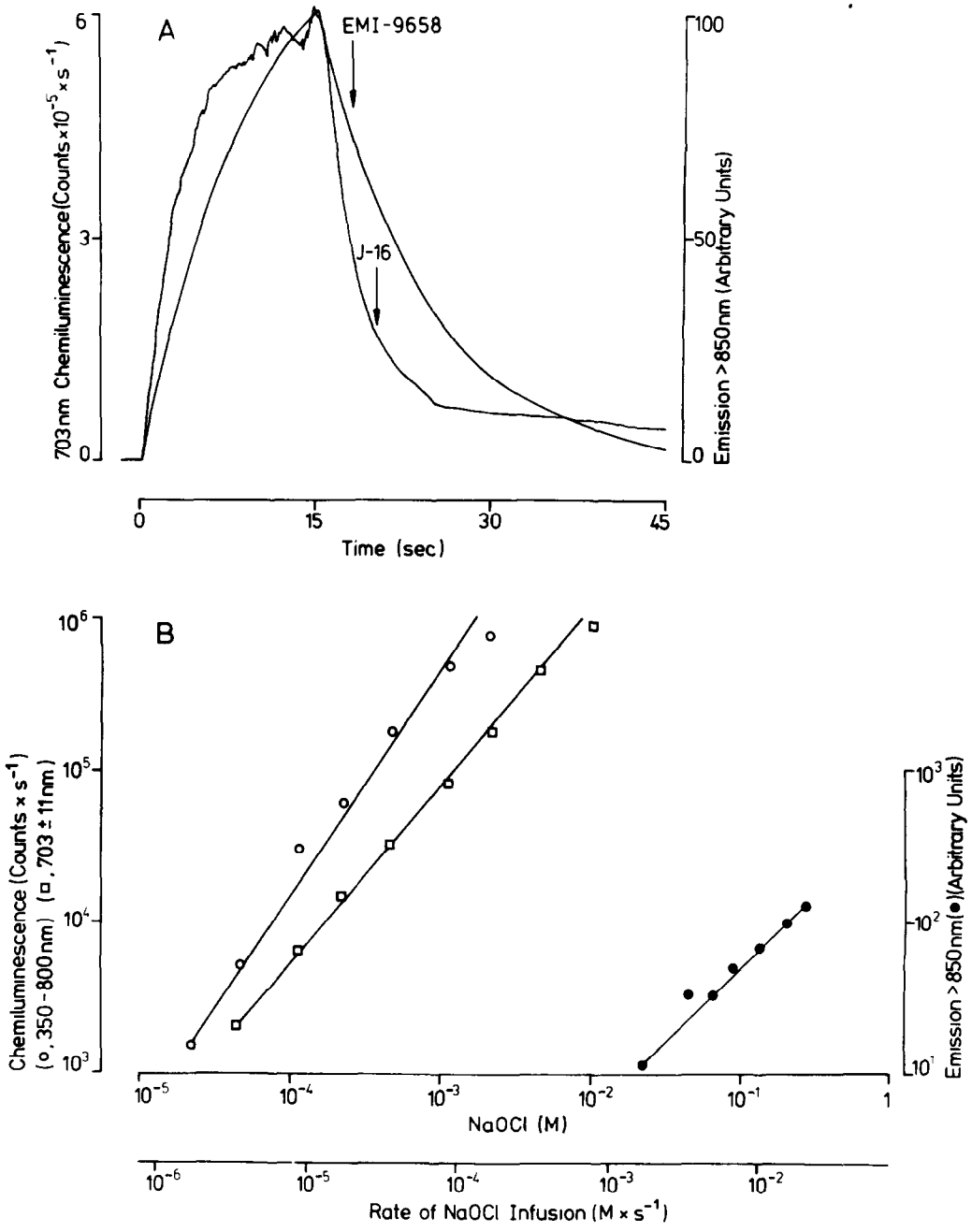

Fig.1. Monomol and dimol emission from the H_2O_2– OCl^- system. Assay conditions as described in section 2. (A) Time course of light emission arising from the H_2O_2–OCl^- system as detected by the red-sensitive photomultiplier (0.6 M NaOCl infused at a rate of 9.2 $\mu l \cdot s^{-1}$ into 7 ml 1.8 M aqueous solution of H_2O_2) and the germanium diode (0.6 M NaOCl infused at a rate of 45 $\mu l \cdot s^{-1}$ into 2.0 ml of 1.8 M H_2O_2). (B) Comparison of sensitivity of the employed red-sensitive photomultiplier (○, □) and germanium diode (●) for the H_2O_2–OCl^- chemiluminescence reaction. EMI-9658, photomultiplier; J-16, germanium diode.

effect of DABCO on singlet oxygen monomol and dimol emission. To this purpose, the H_2O_2–OCl^- system was utilized as a known source of singlet oxygen [2–5].

2. MATERIALS AND METHODS

2.1. *Generation of singlet oxygen by the H_2O_2–OCl^- system*

Singlet oxygen monomol and dimol emission were observed from the H_2O_2–OCl^- reaction as described in [2–5]

$$H_2O_2 + OCl^- \rightarrow Cl^- + H_2O + {}^1O_2 \quad (1)$$

1.8 M H_2O_2 in water was placed in a glass cuvette and NaOCl (0.6 M) was added to the hydroperoxide solution through polyethylene tubing with either a peristaltic pump or a digital precision injection pump. Infusion rates ranged between 0.001 and 1.1 $ml \cdot min^{-1}$.

2.2. *Singlet oxygen dimol emission (634 and 703 nm) measurements*

Light emission was detected as in [11,12] by a red-sensitive photomultiplier (EMI 9658 AM; EMI-Gencom, Plainview, NY) with an applied potential of −1.1 kV and cooled to −25°C by a thermoelectric cooler (model FACT 50 MK III; EMI-Gencom). The photomultiplier was connected to an amplifier-discriminator (model 1121A; EG&G Applied Research, Princeton, NJ) adjusted for single-photon counting. The amplifier-discriminator output was fed into both a frequency counter and a recorder. Chemiluminescence measurements were carried out in a 35 × 5 × 56 mm glass cuvette provided with a lid for tubing connections, which permitted additions from outside of a light-tight box. Constant stirring was assured during the reaction period.

Spectral analysis from the H_2O_2–OCl^- system chemiluminescence was carried out in the 600–750 nm range with interference filters (Jenaer Glaswerk, Schott, Mainz, and Balzers AG, Geisenheim) with maximal transmittance (between 42.1 and 57%) at 599, 620, 634, 649, 654, 668, 694, 703, 708 and 752 nm; bandwidth ranged between 8.1 and 19.1 nm. Light transmitted by these filters was corrected for peak transmission of each filter and spectral response of the photomultiplier. The interference filters were placed in a filter holder between the cuvette and a lucite rod [polymethyl-(methacrylate)], which served as thermal insulator and optical coupler to the photomultiplier.

2.3. *Singlet oxygen monomol emission (1270 nm) measurements*

Infrared emission of singlet oxygen was measured with a germanium detector (type J-16, Judson Infrared, Inc., Ft. Washington, PA) cooled to −30°C and with an active area of 25 mm^2. The sensitivity of this detector at 1270 nm was:

$$D_\lambda^*(\lambda, 900, 1) = 6 \times 10^{10}\,cm \cdot Hz^{1/2} \cdot W^{-1}.$$

The detector signal was intensified with a Burr–Brown amplifier, which was connected to a recorder. Wavelength of chemiluminescence was assessed with IR-PAL interference filters (Jenaer Glaswerk, Schott, Mainz) at 1251, 1261, 1274, 1285 and 1289 nm. Emission spectrum was corrected for spectral response of the germanium diode and transmission values of the filters. Reactions were carried out in a 2-ml cuvette with mirrored walls in order to increase light reflection.

2.4. *Chemicals*

H_2O_2 was obtained from Merck (Darmstadt) and DABCO from Aldrich Chemical Co. (Milwaukee, WI).

3. RESULTS AND DISCUSSION

3.1. *Singlet oxygen monomol and dimol emission. Its dependence on NaOCl concentration*

NaOCl was infused at different rates during 15 s into a 1.8 M H_2O_2 aqueous solution. After this period the infusion pump was turned off and decay of light intensity was followed during further 30–45 s. Fig.1A shows typical time courses of light emission detected with the photomultiplier at 703 nm and the germanium diode (beyond 850 nm). Fig.1B compares detection sensitivity of the red-sensitive photomultiplier and the germanium diode for the H_2O_2–OCl^- reaction in terms of OCl^- concentration and infusion rate. The arbitrary detection limit is defined as 10^3 counts $\cdot s^{-1}$, and for the 703 nm chemiluminescence detected with the photon-counting apparatus was observed with 25 μM NaOCl (theoretical final concentration in-

fused during the 15 s period). The detection limit with the germanium diode, however, was observed to be above about 2 mM NaOCl (this lower limit is not shown in fig.1B, and would correspond to about 0.5 planimetric units).

Since the amounts of singlet oxygen generated in biological systems would be minute, a 1000-fold higher sensitivity by the red-sensitive photomultiplier than the germanium diode offers a better chance to detect this particular light emission. However, we are aware that this argument focusses only on the technical limitations for detection of singlet oxygen luminescence; clearly, a larger active area of the germanium diode would increase its sensitivty. A moderate efficiency of singlet oxygen dimol emission is limited by the requirement of bimolecular collision between short-lived molecules [10^{-6} s for $O_2(^1\Delta g)$]. Recently, 1270 nm chemiluminescence was reported to be produced during the lactoperoxidase/halide-catalyzed decomposition of H_2O_2 [13]. Disregarding the large amounts of enzyme and substrate (1.3–8.7 μM and 20 mM, respectively) necessary to achieve a detectable signal at 1270 nm, a spectral analysis of light detected between 300–800 nm (in a positive case to be attributed to singlet oxygen dimol emission) is unfortunately missing in this report [13]; this precludes, of course, a comparison of monomol and dimol emission in this biological reaction in terms of detectability, reliability, and efficiency.

3.2. *Effect of DABCO on singlet oxygen monomol and dimol emission*

Since the DABCO-stimulated singlet oxygen dimol emission described in [4,5] could be accompanied by an inhibition of singlet oxygen monomol emission, we investigated this possibility. Interestingly, the converse is the case: DABCO enhanced the 1274 nm chemiluminescence of the H_2O_2–OCl^- reaction by about 20% (fig.2), as detected by the germanium diode. The spectral characteristics of dimol emission (in agreement with [4,5]) were not changed by DABCO (fig.3). Although an inhibition of monomol emission by DABCO is not observed, the tentative ratios of monomol/dimol emission are lowered in the presence of the tertiary amine (table 1).

The results shown here concerning dimol emission of singlet oxygen and the effect of DABCO on

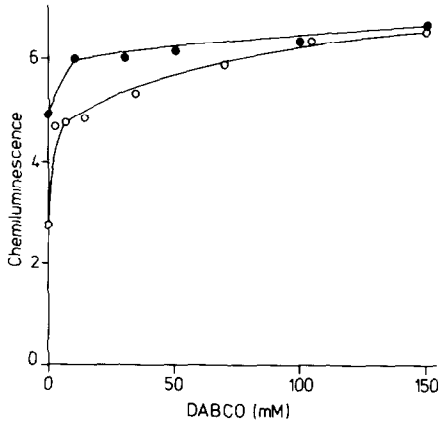

Fig.2. Enhancement of monomol and dimol emission by DABCO. Assay conditions: 0.6 M NaOCl was infused at a rate of 45 $\mu l \cdot s^{-1}$ and 3.6 $\mu l \cdot s^{-1}$ for monomol and dimol emission experiments, respectively, in the presence of increasing concentrations of DABCO. Values in the *y*-axis indicate 703 nm emission (○) detected by the red-sensitive photomultiplier expressed in counts · s^{-1} × 10^{-5}; (●) 1274 nm chemiluminescence expressed in arbitrary units.

it reproduce the observations in [4,5], and they are used in this context in order to make the comparisons with monomol emission under similar experimental conditions. In addition, fig.3 also shows that the light emitted by the H_2O_2–OCl^- system in

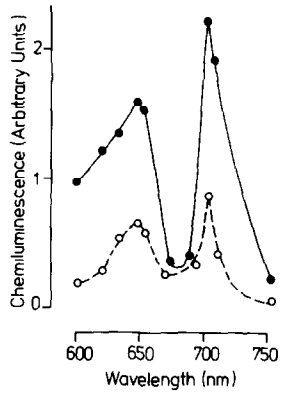

Fig.3. Spectral analysis of red-band chemiluminescence from the H_2O_2–CCl^- system in the absence (○) and presence (●) of DABCO. NaOCl was infused at a rate of 3.6 $\mu l \cdot s^{-1}$ during 15 s (total NaOCl infused 4.7 mM) into 7.0 ml of 1.8 M H_2O_2. DABCO, when present, 100 mM. Values are corrected for spectal response of the photomultiplier and spectral characteristics of the filters.

Table 1

Effect of DABCO on singlet oxygen monomol and dimol emissions

	No additions	+100 mM DABCO
	Photoemission (arbitrary units)	
Monomol emission		
1270 nm	15.4	19.2
Dimol emission		
634 nm	5.4	13.5
668 nm	0.5	0.7
703 nm	8.5	22.0
703/634 ratio	1.57	1.63
Monomol/dimol ratio		
1270/634	2.85	1.42
1270/703	1.80	0.87

Assay conditions as described in section 2. Values are expressed in arbitrary units after correction for the photomultiplier and germanium diode spectral response and spectral characteristics of the filters

our photon counting apparatus conforms to that expected for singlet oxygen monomol emission. The present observations show that the DABCO-enhanced singlet oxygen dimol emission is accompanied by a change in the relationship of monomol and dimol emission, in spite of a concomitant increase of monomol emission intensity by DABCO.

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