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Determination of chemical rate constants in singlet molecular oxygen reactions by using 1,4-dimethylnaphthalene endoperoxide

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Abstract

Thermal decomposition of 1,4-dimethylnaphthalene endoperoxide (DMNE) as a source of singlet oxygen was used to measure chemical rate constants, k_R, for reactions between singlet oxygen, $O_2(^1\Delta_g)$, and various substrates. Time resolved $O_2(^1\Delta_g)$ IR luminescence detection and steady-state experiments were used to monitor the decomposition of the endoperoxide and the rate of singlet oxygen production. Only 25% of oxygen from thermal decomposition of DMNE, in acetonitrile at 20 °C, is detected as $O_2(^1\Delta_g)$. Values of k_R for reactions of $O_2(^1\Delta_g)$ with 1,4-diphenylisobenzofurane (DPBF) and rubrene measured by this method are similar to values obtained by photosensitization. Values of k_R for the chemical reaction of $O_2(^1\Delta_g)$ with the antiinflammatory drugs piroxicam and tenoxicam, of $(6.1 \pm 0.4) \times 10^6$ and $(1.6 \pm 0.2) \pm 10^7$ M^{-1} s^{-1}, respectively, are close to those for the total singlet oxygen deactivation rate. Thermal decomposition of aromatic endoperoxides is a convenient source of singlet oxygen for measurements of rate constants in reactions of $O_2(^1\Delta_g)$ where photosensitization cannot be employed. However, experimental conditions and approaches involved determine the method's limitations and applicability in a given system.

Keywords: Singlet oxygen; Aromatic endoperoxides; Chemical reaction rate constant; Oxicams; Actinometry

1. Introduction

In recent years, reactions of singlet oxygen, $O_2(^1\Delta_g)$, with molecules of biological interest have been studied intensively [1,2] because of the important role of excited oxygen in the oxidative damage of living systems under the simultaneous action of light, ambient oxygen and a sensitizer. The kinetics of singlet oxygen reactions with biological targets can be described in terms of a total rate constant, k_T, that includes both physical and chemical reaction channels [3]. Direct detection of the near-IR phosphorescence of $O_2(^1\Delta_g)$ appears to be the ideal method to evaluate k_T, whereas determination of the chemical reaction rate constant, k_R, normally involves determination of substrate consumption rate in steady-state experiments and comparison with the consumption rate of an actinometer [4]. Photosensitization is the usual method for generating singlet oxygen in these experiments. However, there are experimental difficulties because of long irradiation times and structural complexities of biologically important molecules, mainly related to their polyfunctionality. The most evident problems are spectral overlap, basal state interactions between substrate and sensitizer, excited state interactions between substrate and sensitizer, occurrence of Type I reactions and difficulties in actinometry. Chemical or biochemical routes to generate singlet oxygen, such as the H_2O_2/OCl^- reaction, electron transfer from superoxide anion, or enzymatic reactions work well in aqueous media. However, thermal decomposition of aromatic endoperoxides to the parent arene and molecular oxygen (singlet + triplet), is a valuable non-photochemical source of $O_2(^1\Delta_g)$, widely employed synthetically in organic solvents [5,6], to determine singlet oxygen total quenching rate constants by several substrates [7–13], and used to study singlet oxygen effects on biological media [14–17]. In particular, from the decomposition rate constant and fractional yield of singlet oxygen, DMNE in dioxane at temperatures near 20 °C generates enough $O_2(^1\Delta_g)$ for kinetic measurements of substrate consumption [18].

This study describes a procedure that employs thermally generated excited oxygen to determine chemical rate constants for reactions of $O_2(^1\Delta_g)$ and a given substrate in systems where photosensitization is not possible. This method has been used to determine k_R for reactions of $O_2(^1\Delta_g)$ with piroxicam and tenoxicam. Determination of chemical reaction rate constants for these non-steroidal antinflammatory

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drugs is relevant in establishing whether the photoproducts from reactions with singlet molecular oxygen are involved in the photoprocesses that causes adverse light-induced biological effects as previously proposed [19,20].

2. Experimental

Piroxicam and tenoxicam (Sigma), Rose Bengal, 1,4-dimethylnaphthalene, rubrene, 1,3-diphenylisobenzofurane (DPBF) and 5,10,15,20-tetraphenyl-21H,23H-porphine (TPP) (Aldrich Chemical Co.) were used without further purification. Methylene blue (Merck) was recrystallized from ethanol. All solvents (Merck) were of spectroscopic or HPLC quality.

UV–VIS absorption spectra and steady-state kinetic experiments were performed in a Unicam UV–4 spectrophotometer. Fluorescence experiments were carried out in a Spex Fluorolog 2 Tau 2 spectrofluorimeter. Cell holders were kept at $20 \pm 0.5\,^{\circ}C$ by water from a Haake thermoregulated bath.

1,4-Dimethylnaphthalene endoperoxide (DMNE) was synthesized as described [18]; 0.5 ml of distilled 1,4-dimethylnaphthalene, 2.0 mg of methylene blue and 10 ml of methylene chloride were irradiated with a 50 W halogen lamp for 20 h at $1.0 \pm 0.1\,^{\circ}C$. Methylene blue was removed from the resulting bluish solution by using an alumina column. The colorless solution obtained by elution with methylene chloride was dried in vacuum, at below $1.0\,^{\circ}C$. From the resulting mixture, i.e. the white solid product and a colorless liquid (1,4-dimethylnaphthalene), the endoperoxide was isolated by filtration. Pure DMNE was obtained as white shiny needles by recrystallization from hexane:dichloromethane 10:1 in 40% yield.

Initial chemical rate constants for reactions of $O_2(^1\Delta_g)$ with piroxicam, tenoxicam, rubrene and DPBF were determined as follows. Fresh acetonitrile solutions of DMNE (0.8–6 mM) and appropriate concentrations of substrate (10^4 to 10^{-5} M) in 1 cm spectrophotometer cuvettes were kept at $20 \pm 0.5\,^{\circ}C$ in a spectrophotometer cell holder. Substrate consumption was monitored by its absorbance decrease. Concentrations in the order of 10^{-7} M were used with DPBF and its consumption was monitored by its fluorescence emission at 455 nm. Solutions of DPBF freshly prepared in the dark were used. Under our conditions, auto-oxidation of DPBF was lower than 1%.

Time resolved phosphorescence measurements were carried out in 1 cm-path fluorescence cuvettes. TPP excitation was by absorption of 500 ps light pulses of a PTI model PL-202 dye laser (419 nm, ca. 200 μJ per pulse). A PTI model PL-2300 nitrogen laser was used to pump the dye laser. Rose Bengal excitation was by absorption of 6 ns light pulses of a Quantel Brilliant Nd-Yag laser (532 nm, ca. 4 mJ per pulse). A liquid N_2-cooled North Coast model EO-817P germanium photodiode detector with a built-in preamplifier was used to detect infrared radiation emitted from the cuvette. The detector and the cuvette were fitted at a right-angle geometry. An interference filter (1270 nm, Spectrogon US Inc.) and a cut-off filter (995 nm, Andover Corp.) were the only elements between the cuvette face and the diode cover plate. Preamplifier output was fed into the 1 MΩ input of a Hewlett Packard model 54540 A digitizing oscilloscope. Computerized experiment control, data acquisition and analysis were performed with LabView-based software developed in our laboratory.

3. Results and discussion

Eqs. (1)–(5) describe all kinetically significant processes of generation, decay and reactions of singlet oxygen involved in thermal cycloreversion of aromatic endoperoxides in the presence of substrate, S:

$$ArO_2 \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} Ar + {}^1O_2 \tag{1}$$

$${}^1O_2 \xrightarrow{k_\Delta} O_2 \tag{2}$$

$${}^1O_2 + Ar \xrightarrow{k'_q} {}^3O_2 + Ar \tag{3}$$

$${}^1O_2 + S \xrightarrow{k_R} \text{products} \tag{4}$$

$${}^1O_2 + S \xrightarrow{k_q} {}^3O_2 + S \tag{5}$$

where k_1 accounts for endoperoxide decomposition; k_Δ, singlet oxygen deactivation by solvent; k'_q, singlet oxygen quenching by the aromatic molecule, Ar, produced by endoperoxide decomposition; k_R, chemical reaction of singlet oxygen with S; and k_q, singlet oxygen quenching by substrate. The endoperoxide decomposition that generates triplet oxygen was omitted because it does not contribute to substrate disappearance.

According to Eqs. (1)–(5), the substrate consumption is given by Eq. (6):

$$-\frac{\partial[S]}{\partial t} = \frac{k_1 k_R [ArO_2][S]}{(k_{-1} + k_q)[Ar] + k_\Delta + (k_q + k_R)[S]} \tag{6}$$

With experiments at low endoperoxide conversion or by taking initial rates, all processes depending on concentration of Ar, i.e. the reverse reaction in Eq. (1) and quenching of singlet oxygen, Eq. (3), can be disregarded and the substrate consumption rate is given by Eq. (7):

$$-\left(\frac{\partial[S]}{\partial t}\right)_0 = \frac{k_1 k_R [ArO_2]_0 [S]_0}{k_\Delta + k_T [S]_0} \tag{7}$$

where $k_T = k_Q + k_R$ and subscript "0" indicate initial rate and concentration. Eq. (7) takes a similar form to the equation employed to account for substrate consumption rates in classical photosensitizations [21].

Rate constants for thermal decomposition of DMNE, k_{desc}, were determined by measurement of the naphthalene

absorption at 283 nm. A value of k_{desc} of $(5.0 \pm 0.2) \times 10^{-5}\,s^{-1}$ was obtained for endoperoxide decomposition in acetonitrile at 20 °C.

The rate constant of singlet oxygen generation from endoperoxide, k_1, was determined from the consumption rate of a substrate that reacts very efficiently with singlet oxygen through the chemical pathway, and can trap all excited oxygen generated in the medium. 9,10-diphenylisobenzofuran [22,23] and α-terpinene [24] meet this requirement, and we used the former. For singlet oxygen decay in acetonitrile k_Δ is $(1.2 \pm 0.04) \times 10^4\,s^{-1}$ (averaged over several determinations in our laboratory). In addition, reported values for the total rate constant of $O_2(^1\Delta_g)$ quenching by DPBF are $\approx 10^9\,M^{-1}\,s^{-1}$ [25]. Thus, at high [DPBF], $k_\Delta \ll (k_q + k_R)$ [S]. Since for DPBF $k_T = k_R$, at high [DPBF], its consumption rate depends only on the rate of singlet oxygen generation from endoperoxide. Thus, Eq. (7) simplifies to Eq. (8):

$$-\left(\frac{\partial[S]}{\partial t}\right)_0 = k_1[ArO_2]_0 \quad (8)$$

Fig. 1 shows the dependence of the initial rate of DPBF consumption followed spectrophotometrically at 410 nm on the initial endoperoxide concentration according to Eq. (8). Initial [DPBF] between 3.5×10^{-5} and 1.1×10^{-4} M were used because in this range DPBF consumption rates depend only on the initial endoperoxide concentration. From the plot in Fig. 1, the singlet oxygen generation rate constant, $k_1 = (1.26 \pm 0.04) \times 10^{-5}\,s^{-1}$.

In addition, Eq. (9) accounts for substrate consumption rates when $k_\Delta \gg (k_r + k_q)$ [S]

$$-\left(\frac{\partial[S]}{\partial t}\right)_0 = \frac{k_1 k_R [ArO_2]_0 [S]_0}{k_\Delta} \quad (9)$$

Eq. (9), shows that the initial substrate consumption is a second-order process, dependent on initial [DPBF]. A bimolecular transfer of oxygen from endoperoxide to the substrate to give the expected products, Eq. (10), can be considered [6]:

$$ArO_2 + S \xrightarrow{k_{bim}} \text{products} \quad (10)$$

The rate of this process is proportional to concentrations of DMNE and acceptor, Eq. (11):

$$-\frac{\partial[S]}{\partial t} = k_{bim}[ArO_2][S] \quad (11)$$

and substrate consumption fits a second-order rate equation. Fig. 1 shows experimental points obtained at different [DPBF] concentrations and the same initial [DMNE]. The DPBF disappearance is independent of initial [DPBF], indicating that k_Δ is at least one order of magnitude smaller than $(k_r + k_q)$ [S] as required by Eq. (8). Additionally, this result rules out a second-order process involving direct oxygen transfer from endoperoxide to the acceptor.

From the ratio between the rate constant of endoperoxide decomposition, k_{desc}, and the rate constant of singlet oxygen generation, k_1, we show that in acetonitrile at 20 °C only 25% of molecular oxygen generated by thermal decomposition of DMNE is $O_2(^1\Delta_g)$. This ratio is much lower than that reported by Turro et al. [18] who found that singlet oxygen formation ranged between 50 and 73% in dioxane at 60 °C.

Eq. (9) should be used to determine substrate consumption rates with less reactive substrates ($k_T < 10^7\,M^{-1}\,s^{-1}$) or at low [substrate] or both. These conditions were used in measuring k_R values for reactions of singlet oxygen with the antiinflammatory drugs piroxicam and tenoxicam. All attempts at measuring k_R by photosensitization were

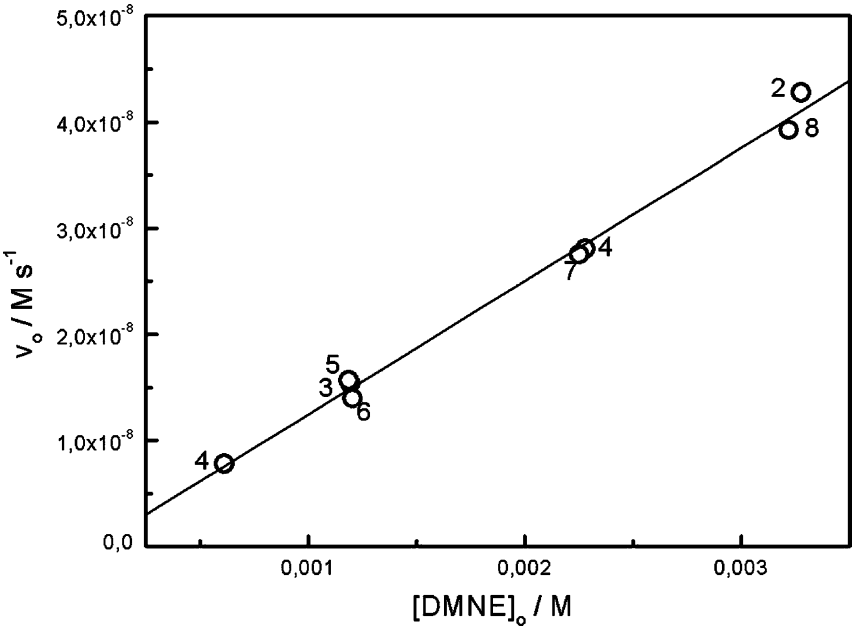

Fig. 1. Plot of initial rate of substrate consumption versus the product of the initial concentrations of substrate and endoperoxide for reaction of $O_2(^1\Delta_g)$ with piroxicam (□), tenoxicam (○) and rubrene (△) in acetonitrile at 20 °C.

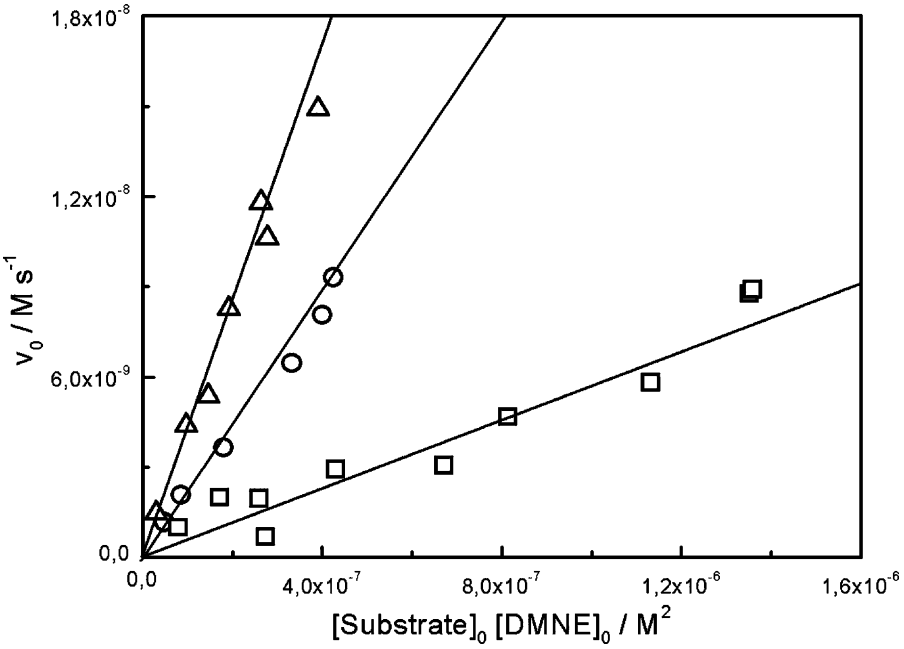

Fig. 2. Dependence of initial rates of DPBF consumption on initial [DMNE] for the reaction between $O_2(^1\Delta_g)$ and DPBF in acetonitrile at 20 °C. $[DPBF]_0$ = 1: 6.7×10^{-5} M; 2: 6.4×10^{-5} M; 3: 7.0×10^{-5} M; 4: 6.7×10^{-5} M; 5: 8.7×10^{-5} M; 6: 3.5×10^{-5} M; 7: 1.1×10^{-4} M; 8: 1.3×10^{-5} M.

unsuccessful with these compounds [26]. We also determined k_R for rubrene and DPBF to test the reliability of the method. As previously stated, the latter compound reacts very efficiently with $O_2(^1\Delta_g)$, it is then necessary to employ very low [DPBF] to satisfy Eq. (9). Here, fluorescence emission at 455 nm was used to monitor consumption rates.

Fig. 2 shows data obtained in acetonitrile for piroxicam, tenoxicam and rubrene, from initial rates, plotted according to Eq. (9). Results obtained for DPBF are not included in this plot because of the high slope. Values of k_R for all the compounds, calculated from slopes of these plots are in Table 1.

The chemical rate constant for reaction between rubrene and $O_2(^1\Delta_g)$ is lower than the total rate constant previously reported ($k_T = 8.0 \times 10^7\ M^{-1}\ s^{-1}$) [27] and that determined by time resolved experiences in our laboratory ($(k_T = 1.0 \pm 0.04) \times 10^8\ M^{-1}\ s^{-1}$). Similar differences between k_T and k_R for reactions of $O_2(^1\Delta_g)$ with rubrene have been reported in benzene [27,28] and CCl_4 [29]. For DPBF the value of k_R, $(1.1 \pm 0.05) \times 10^9\ M^{-1}\ s^{-1}$, is very close to that of k_T, $(1.13 \pm 0.05) \times 10^9\ M^{-1}\ s^{-1}$, from time resolved experiments. These values, are in the previously reported range ($(1.0–1.4) \times 10^9\ M^{-1}\ s^{-1}$) [25]. The reaction rate constants for both, piroxicam and tenoxicam are close to those for total singlet oxygen deactivation by oxicams, $(6.4 \pm 0.5) \times 10^6$ and $(1.3 \pm 0.2) \times 10^7\ M^{-1}\ s^{-1}$ for piroxicam and tenoxican, respectively [26]. These results indicate that for this type of ene-reaction [30], exciplex formation in acetonitrile is the rate determining step in accordance with the proposition of Adam et al. [31] for reaction of singlet oxygen with electron rich olefins (Schenk reaction). In order to exclude eventual bimolecular transfer of oxygen from endoperoxide to the oxicams, a process that cannot be disregarded in the kinetic treatment, we monitored substrate consumption in presence of DPBF to reduce singlet oxygen concentration. DPBF was selected because it does not react through a bimolecular direct oxygen transfer, as previously demonstrated. Due to overlap between spectra of oxicams and DPBF, time dependent substrate absorbance was obtained by deconvolution of the absorption spectrum. Fig. 3 shows data obtained for oxidation of Piroxican by singlet oxygen thermally generated in the presence and absence of DPBF. These plots, show that substrate consumption is dependent on singlet oxygen concentration. Piroxicam concentration remains nearly constant (or diminishes very slowly) in the presence of DPBF at the beginning of the reaction. As concentration of the furan derivative is lowered, because it reacts more rapidly with $O_2(^1\Delta_g)$ than piroxicam, the drug consumption rate increases appreciably. Then, the contribution of direct bimolecular oxygen transfer to oxicam oxidation can be ruled out.

Table 1
Chemical rate constants ($M^{-1}\ s^{-1}$) in acetonitrile at 20 °C, determined by using thermal cycloreversion of DMNE as $O_2(^1\Delta_g)$ source

Piroxicam	Tenoxicam	Rubrene	DPBF
$(6.1 \pm 0.4) \times 10^6$	$(1.6 \pm 0.2) \times 10^7$	$(3.8 \pm 0.2) \times 10^7$	$(1.1 \pm 0.05) \times 10^9$

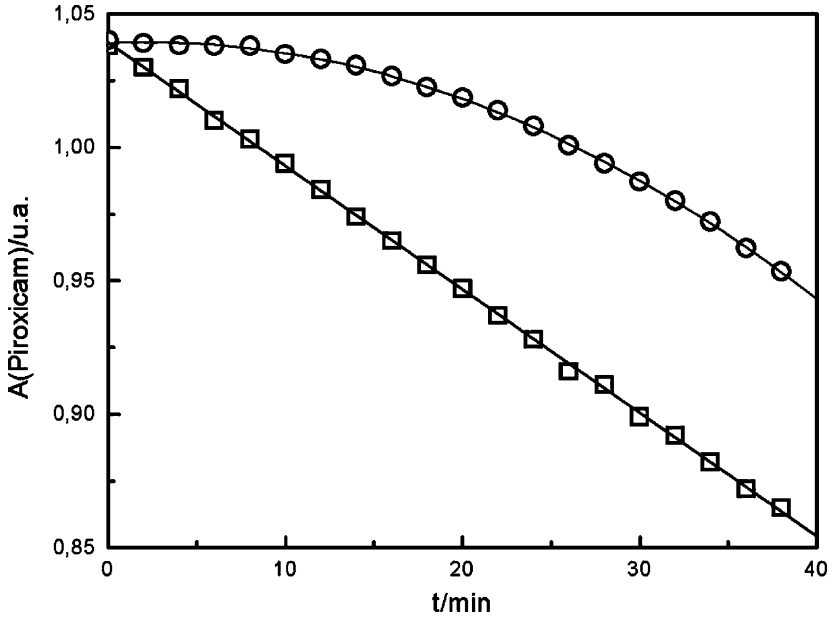

Fig. 3. Plot of piroxicam consumption vs. t for reaction with $O_2(^1\Delta_g)$ generated from DMNE in acetonitrile at 20 °C. Data obtained in the presence (○) and absence (□) of DPBF. $[DPBF]_0 = 5 \times 10^{-5}$ M; $[piroxicam]_0 = 5 \times 10^{-5}$ M; $[DMNE]_0 = 2.8 \times 10^{-3}$ M.

In summary, thermal decomposition of aromatic endoperoxides is a convenient source of singlet molecular oxygen for measurements of chemical rate constants for reactions between $O_2(^1\Delta_g)$ and organic substrates, mainly polyfunctional molecules of biological interest, when photosensitization is not suitable. However, precautions must be taken to choose appropriate experimental conditions and the kinetic equations that describe behavior of the system. In addition, this method, that evaluates chemical rate constants for singlet oxygen reactions, avoids use of an actinometer, as in photosensitization procedures.

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