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Journal of Photochemistry and Photobiology A: Chemistry 195 (2008) 1–9
Journal of Photochemistry and Photobiology A:Chemistry
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Supramolecular carriers of singlet oxygen: Photosensitized formation and thermal decomposition of endoperoxides in the presence of cyclodextrins

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Received 18 June 2007; received in revised form 31 July 2007; accepted 2 September 2007
Available online 12 September 2007

Abstract

Water-soluble 2,7-disulfonato-9,10-diphenylanthracene (2,7-dsDPA) and 2,6-disulfonato-9,10-diphenylanthracene (2,6-dsDPA) react with singlet oxygen $O_2(^1\Delta_g)$ produced by photosensitized reaction and form thermostable endoperoxides. The thermal decomposition of endoperoxides at higher temperatures (60–150 °C) leads to the production of $O_2(^1\Delta_g)$. 2,7-dsDPA, 2,6-dsDPA and corresponding endoperoxides 2,7-dsDPAO_2, 2,6-dsDPAO_2 form 1:1 host–guest complexes with native cyclodextrins (CDs) and 2-hydroxypropylated cyclodextrins (hpCDs). The binding constants (10^2–10^4 M^{-1}) depend on the cavity size and the functionalization of the primary face of CDs. The thermal stabilities of both endoperoxides in host–guest complexes with CDs and hpCDs are highly increased.

Keywords: Singlet oxygen; Diphenylanthracene; Endoperoxides; Cyclodextrins; Host–guest complexes

1. Introduction

Singlet oxygen ($O_2(^1\Delta_g)$), a short-lived, highly oxidative cytotoxic species has received remarkable attention by chemists and biochemists for its interesting mechanistic and synthetic aspects [1–6] and large environmental [7–9], biological and medical significance [10–12]. It is generally assumed that $O_2(^1\Delta_g)$ can be generated *via* photosensitized reactions [13–15]. The mechanism of photosensitized reaction includes the formation of the photosensitizer triplet state and transfer of energy to triplet oxygen leading to $O_2(^1\Delta_g)$ formation [16]. Singlet oxygen can also be produced *via* chemical reactions, e.g. using H_2O_2/ClO^- [17], H_2O_2/MoO_4^{2-} [18] and H_2O_2/CaO_2 [19] systems based on H_2O_2 disproportionation that leads to H_2O and $O_2(^1\Delta_g)$.

Singlet oxygen enters several specific reactions, especially with unsaturated hydrocarbons: [2 + 2] cycloaddition, ene reaction and [4 + 2] cycloaddition [4,5]. [4 + 2] cycloaddition with acyclic, cyclic or aromatic 1, 3-dienes leads to various stable endoperoxides [20]. The thermolysis of many endoperoxides of polycyclic aromatic compounds was suggested to occur *via* the generation of molecular oxygen and parent aromatic species. Chemical reactivity studies have shown that some transannular peroxides produce a large fraction of oxygen in its singlet excited state. Consequently, aromatic endoperoxides can be considered as secondary sources of $O_2(^1\Delta_g)$ *via* thermal decomposition [21,22].

Cyclodextrins (CDs) are water-soluble, cyclic oligosaccharides with 6(α), 7(β) or 8 (γ) D-glucopyranose units, which have a relatively hydrophobic cavity for guest binding. Guest molecules are bonded in the cavity of CDs (host molecules) by a combination of hydrophobic, van der Waals and electrostatic interactions [23–25]. Formation of host–guest complexes with CDs is accompanied by changes of the physical and photophysical properties of the guest molecule. The changes are caused by a

Abbreviations: βCD; γCD; hpCD, (, γ and 2-hydroxypropylated cyclodextrin; DPA, 9,10-diphenylanthracene; 2,6-dsDPA, 2,6-disulfonato-9,10-diphenylanthracene; 2,7-dsDPA, 2,7-disulfonato-9,10-diphenylanthracene; dsDPA and dsDPAO_2, disulfonato derivatives of 9,10-diphenylanthracene and corresponding endoperoxides; TPPS, 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin; $O_2(^1\Delta_g)$, singlet molecular oxygen.

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doi:10.1016/j.jphotochem.2007.09.007

number of factors: limitation of the rotation and torsion motion, limitation of interactions with water protons, protective effects against external quenching, disaggregation caused by CDs, etc. [23,24]. CD complexes frequently improve the resistance of guest molecules to thermal degradation and increase the solubility and biological activity [26,27]. They are worth considering as inert carriers of aromatic $O_2(^1\Delta_g)$ acceptors and corresponding endoperoxides for possible tuning of their physical properties.

9,10-Diphenylanthracene (DPA) is known as a photosensitizer that produces $O_2(^1\Delta_g)$ under irradiation and also represents an efficient $O_2(^1\Delta_g)$ acceptor forming endoperoxide ($DPAO_2$) with 80–100% yield [21,28,29]. $DPAO_2$ is thermostable up to temperature ca 80 °C. Thermal decomposition of $DPAO_2$ at a higher temperature has the character of a reverse reaction to [4 + 2] cycloaddition, leading to the parent compound DPA and oxygen, which is from 32% in its singlet state [21,28,29].

$$DPA + {}^1O_2 \rightleftharpoons DPAO_2 \quad (1)$$

$DPAO_2$ and related aromatic acceptors may be used for controlled release of $O_2(^1\Delta_g)$ in the absence of light.

Here we present the physico-chemical properties of endoperoxides that are formed by [4 + 2] cycloaddition of $O_2(^1\Delta_g)$ with 2,7-disulfonato-9,10-diphenylanthracene (2,7-dsDPA) and 2,6-disulfonato-9,10-diphenylanthracene (2,6-dsDPA) (Fig. 1) in aqueous solution. The study includes synthesis of 2,6-dsDPA and 2,7-dsDPA by sulfonation of DPA followed by recrystallization in different solvents, identification of 2,6-dsDPA and 2,7-dsDPA, preparation of corresponding endoperoxides $dsDPAO_2$s, calculation of stoichiometry and binding constants of host–guest complexes of dsDPAs/$dsDPAO_2$s with native and 2-hydroxypropyl-cyclodextrins (hpCDs), formation of CDs complexes with endoperoxides and the influence of CDs on the properties of the endoperoxides.

2. Experimental

2.1. Materials

Native β-cyclodextrin (βCD, ≥99%), γ-cyclodextrin (γCD, ≥98%), (2-hydroxypropyl)-β-cyclodextrin (hpβCD, molar substitution $f_a = 0.6$), 2-hydroxypropyl-γ-cyclodextrin (hpγCD, molar substitution $f_a = 0.6$; all from Fluka), 9,10-diphenylanthracene (DPA, ≥98%, Merck) and 5, 10, 15, 20-tetrakis(4-sulfonatophenyl) porphyrin (TPPS, Fluka) were used as received. All other chemicals and solvents were of analytical reagent grade. Deionized double-distilled water was used throughout the study.

2.2. Synthesis

Disodium salt of 2,7-disulfonato-9,10-diphenylanthracene (2,7-dsDPA) and disodium salt of 2,6-disulfonato-9,10-diphenylanthracene (2,6-dsDPA) were prepared by sulfonation of 9,10-diphenylanthracene (DPA) using modified method of A. Étienne et al. [30]. 1.0 g DPA was dissolved in 3.5 ml acetic acid and 0.4 ml acetic anhydride. The mixture was cooled in an ice bath. After cooling 1 ml 60% oleum was added and the reaction mixture was refluxed for 1 h at 130 °C. The reaction was stopped by cooling to laboratory temperature. 1.7 g NaCl was added. The mixture was diluted by 15 ml H_2O and warmed to 120 °C until dissolution of NaCl. Subsequent crystallization provided 2,7-dsDPA and 2,6-dsDPA. These products were recrystallized from H_2O (2,7-dsDPA, 240 mg) and from methanol:H_2O (1:1, v/v) mixture (2,6-dsDPA, 290 mg) and identified by NMR spectroscopy. 2,7-dsDPA ^{1}H NMR (399.95 MHz, D_2O), δ_H (ppm): 8.21 (d, 2H, $J = 1.4$ Hz, H-1, H-8), 7.85 (d, 2H, $J = 9.2$ Hz, H-4, H-5), 7.73 (m, 1H, H-4′), 7.72 (m, 2H, H-3′, H-5′), 7.70 (m, 2H, H-3, H-6), 7.66 (m, 1H, H-4″), 7.65 (m, 2H, H-3″, H-5″), 7.51 (m, 2H, H-2′, H-6′), 7.43 (m, 2H, H-2″, H-6″). 2,6-dsDPA ^{1}H NMR (399.95 MHz, D_2O), δ_H (ppm): 8.15 (d, 2H, $J = 1.8$ Hz, H-1, H-5), 7.84 (d, 2H, $J = 9.2$ Hz, H-4, H-8), 7.69 (m, 4H, H-3′,

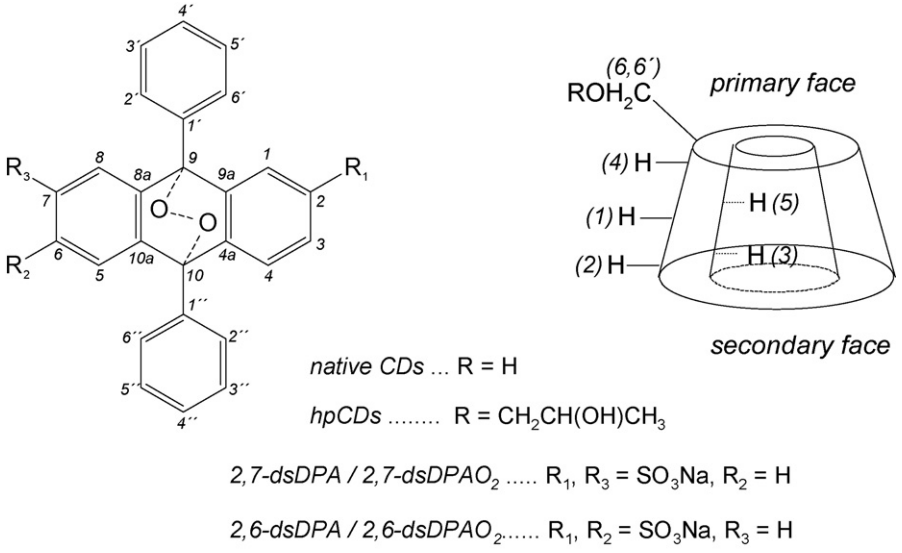

Fig. 1. The schematic structure of dsDPAs and corresponding endoperoxides $dsDPAO_2$ and cyclodextrins. Positions of hydrogen atoms inside and outside of the CDs cavity.

H-5′), 7.68 (m, 2H, H-4′), 7.67 (m, 2H, H-3, H-7), 7.39 (m, 4H, H-2′, H-6).

Endoperoxides 2,7-dsDPAO$_2$ and 2,6-dsDPAO$_2$ were prepared by reaction of 2,7-dsDPA and 2,6-dsDPA with $^1O_2$ generated by photosensitized reaction [13–15]. The preparation was made by irradiating aqueous solutions of dsPDAs (typically 1×10^{-5} M) in the presence of 2×10^{-6} M TPPS on irradiation bench. In a typical experiment, steady state irradiation of the above solution with a photosensitizer in 1 cm × 1 cm closed quartz cell was performed in a thermostated (22 °C) steel chamber fixed to optical bench. The radiation came from a polychromatic 250 W stabilized halogen lamp. The decrease of absorption bands of dsDPAs at $\lambda_{max} = 268$ nm was monitored. Endoperoxides were identified by NMR spectroscopy. 2,7-dsDPAO$_2$, ^{1}H NMR (399.95 MHz, D_2O), δ_H (ppm): 7.79 (dd, 2H, $J_1 = 8.1$ Hz, $J_2 = 1.8$ Hz, H-3, H-6), 7.76 (m, 2H, H-2′, H-6′), 7.68–7.80 (m, 2H, H-3′, H-5′), 7.68–7.80 (m, 1H, H-4′) 7.67 (d, 2H, $J = 1.8$ Hz, H-1, H-8), 7.61 (m, 2H, H-2″, H-6″), 7.57–7.59 (m, 2H, H-3″, H-5″), 7.57–7.59 (m, 1H, H-4″), 7.37 (d, 2H, $J = 8.1$ Hz, H-4, H-5). 2,6-dsDPAO$_2$, ^{1}H NMR (399.95 MHz, D_2O), δ_H (ppm): 7.77 (dd, 2H, $J_1 = 8.1$ Hz, $J_2 = 1.8$ Hz, H-3, H-7), 7.72 (m, 4H, H-2′, H-6′), 7.72 (m, 4H, H-3′, H-5′), 7.69 (m, 2H, H-4′), 7.65 (d, 2H, $J = 1.8$ Hz, H-1, H-5), 7.41 (d, 2H, $J = 8.1$ Hz, H-4, H-8).

2.3. Detection of $O_2(^1\Delta_g)$

The $O_2(^1\Delta_g)$ formation was followed by direct observation of the time resolved luminescence at 1270 nm and using the iodide method based on the reaction of $O_2(^1\Delta_g)$ with I^- in the presence of $(NH_4)_2MoO_4$ as a catalyst [31]. The amount of (photo)produced I_3^- is directly proportional to the concentration of O_2 ($^1\Delta_g$) that is produced during continuous irradiation or dark reaction. The mechanism of I^- oxidation by O_2 ($^1\Delta_g$) is as follows [31,32]:

$$^1O_2 + I^- \rightarrow IOO^- \xrightarrow{H_2O} IOOH \xrightarrow{I^-} I_2OOH^- \rightarrow I_2 + HO_2^- \xrightarrow{I^-,H_2O} I_3^- + H_2O_2 + OH^- \quad (2)$$

$$H_2O_2 + 2I^- + 2H^+ \rightarrow I_2 + 2H_2O \quad (3)$$

$$I_2 + I^- \rightarrow I_3^- \quad (4)$$

The following composition of iodide detection solution for $O_2(^1\Delta_g)$ was used: 0.1 M KI, 10 μM $(NH_4)_2MoO_4$ in 0.02 M sodium–potassium phosphate buffer, pH 6.2. To detect $^1O_2$ photogenerated *via* photosensitized reaction, 2 ml of an iodide detection solution containing dsDPA were placed into a thermostated 10 mm quartz cell (22 °C) and irradiated by a 250 W stabilized halogen lamp. The solution was stirred during irradiation. The increasing absorbance of the I_3^- band was recorded at 287 and 351 nm and compared with a blank solution of the same composition kept in the dark.

2.4. Methods

The stoichiometry of the dsDPA/CD complexes was determined by Job's method of continuous variations [33]. The solution of dsDPA and each corresponding CDs were mixed to reach a standard volume while the total concentration of both components remained constant, i.e 1×10^{-5} M. Job's plots were constructed from the absorbance differences in the absorption maxima of the complexes at 233 nm. An equilibrium between free (unbound) and bound form of dsDPA in supramolecular complex with CD can be expressed by Eq. (5):

$$\text{dsDPA} + n\text{CD} \rightleftharpoons \text{dsDPA-CD}_n \quad (5)$$

The binding constant K_b is defined by the expression

$$K_b = \frac{[\text{dsDPA-CDn}]}{((\text{CD}_T\text{-}n[\text{dsDPA-CD}_n])^n[\text{dsDPA}])}, \quad (6)$$

where [dsDPA-CD$_n$] and [dsDPA] are the equilibrium molar concentrations of the complex and the free dsDPA, respectively, CD_T is the total molar concentration of CD and n stands for the stoichiometry. The binding isotherms were constructed from a set of 16–26 solutions containing dsDPA (1×10^{-5} M) and cyclodextrins in a range of concentrations from 2×10^{-4} to 1×10^{-2} M. The binding isotherms for the formation of 1:1 complexes were analyzed using the hyperbolic relationship between the observed change of absorbance ($\Delta A = A - A_o$) and the equilibrium free molar concentration of cyclodextrin [CD]:

$$\Delta A = \frac{\Delta A_\infty K_b[\text{CD}]}{(1 + K_b[\text{CD}])}, \quad (7)$$

where $\Delta A_\infty = A_\infty - A_o$; A_o, A and A_∞ are the absorbances at 233 nm in the absence of CD, in the presence of CD and of the complex. The binding constants K_b were obtained by non-linear regression [34]. Unless otherwise stated, binding experiments were performed in 0.02 M phosphate buffer (pH 7.0) at room temperature (22 °C).

The time course of thermal decomposition of dsDPAO$_2$s was followed by UV–vis spectrophotometry. Aqueous solutions of dsDPAO$_2$s in sealed glass ampoules were heated to the required temperature in a thermostated oven. Reverse reactions leading to parent dsDPAs were followed after cooling to room temperature at 220–300 nm, the generation of singlet oxygen was followed similarly using the iodide detection solution (see Section 3.3).

2.5. Instrumentation

The UV–vis absorption spectra and fluorescence spectra were measured on a Varian Cary IE spectrophotometer and on a Perkin Elmer LS 50B luminescence spectrometer, respectively, using 10 mm quartz cells.

A Lambda Physik COMPEX 102 excimer laser ($\lambda_{exc} = 308$ nm, pulse length 28 ns, fluence 2.1 mJ cm^{-2}) was used to generate the triplet states and singlet oxygen. Time-resolved near-infrared emission at 1270 nm of $O_2(^1\Delta_g)$ was monitored with a Ge diode (Judson J16-8SP-R05M-HS) in conjunction with an interference filter.

NMR spectra were recorded on a Varian-Inova (400 MHz) instrument using a 5 mm broadband probe or 5 mm gradient inverse probe. All samples were measured in D_2O using 2-

Table 1
Absorption maxima λ_{max}, absorption molar coefficients ε_{max} at λ_{max}, stoichiometry and binding constants K_b of dsDPA/CD complexes calculated using binding isotherm analysis

System	λ_{max} (nm)	ε_{max} (M^{-1} cm^{-1})	Stoichiometry	K_b (M^{-1})[a]
2,7-dsDPA	268.0	1.09×10^5	–	
2,7-dsDPA/hpγCD	270.5	1.12×10^5	1: 1	7.7×10^2
2,7-dsDPA/γCD	269.0	1.09×10^5	1: 1	1.2×10^2
2,7-dsDPA/hpβCD	271.5	1.12×10^5	1: 1	1.3×10^4
2,7-dsDPA/βCD	269.0	1.00×10^5	1: 1	1.4×10^2
2,6-dsDPA	268.0	1.12×10^5	–	–
2,6-dsDPA/hpγCD	270.5	1.13×10^5	1: 1	4.0×10^3
2,6-dsDPA/γCD	270.0	1.13×10^5	1: 1	2.6×10^3
2,6-dsDPA/hpβCD	270.0	1.12×10^5	1: 1	6.6×10^2
2,6-dsDPA/βCD	268.0	1.12×10^5	–[b]	–[b]

All measurements were performed in 0.02 M phosphate buffer at pH 7.0.
[a] Average value from three independent experiments; the non-linear regression fits variation coefficients < ± 10%.
[b] No spectral evidence of complex formation.

methylpropan-2-ol as an internal reference (δ 1.25 ppm for ^{1}H spectra) at 35 °C.

3. Results and discussion

3.1. Characterization of dsDPAs as photosensitizers

It is known that anthracene chromophore absorbs UV light and can produce $O_2(^1\Delta_g)$ under irradiation [35,36]. dsDPAs have strong absorption bands at 268 nm in UV–vis spectrum (Table 1). The ability of dsDPAs to form $O_2(^1\Delta_g)$ was proved by direct observation of $O_2(^1\Delta_g)$ luminescence at 1270 nm and by the iodide method.

Time resolved luminescence measurements (Fig. 2) clearly indicate the ability of dsDPAs to generate $O_2(^1\Delta_g)$. The quantum yields of singlet oxygen Φ_Δ were estimated by a comparative method using 5, 10, 15, 20-tetrakis(4-sulfonatophenyl) porphyrin (TPPS) as a standard ($\Phi_\Delta = 0.62$ in D_2O) [37] using optically matched solutions. The luminescence decays were fitted within 20–200 μs using a monoexponential function and extrapolated to time = 0. Comparison of the signal amplitudes gives $\Phi_\Delta = 0.08$ for both 2,6-dsDPA and 2,7-dsDPA.

Iodide method was used as an alternative method for detection of $O_2(^1\Delta_g)$. The concentration of photoproduced I_3^- is proportional to the concentration of $O_2(^1\Delta_g)$. This is documented by a linear increase of the concentration of I_3^- produced by dsDPAs during continuous irradiation (Fig. 3a and Fig. 3 inset). The reaction solution was bubbled with N_2 during the irradiation to eliminate air oxygen and to prove that photooxidation of I^- is caused by oxygen species. Under these conditions, no increasing absorption bands of I_3^- were found. The importance of $O_2(^1\Delta_g)$ for the formation of I_3^- was further tested in D_2O and using the singlet oxygen quencher NaN_3. A marked acceleration of the formation of I_3^- (Fig. 3b) fully corresponds to a higher efficiency of $O_2(^1\Delta_g)$ to oxidize I^- to I_3^- in D_2O since the lifetime of $O_2(^1\Delta_g)$ is much longer in D_2O (40–80 μs) than in H_2O (2–4 μs). In air-saturated solutions containing NaN_3, no production of I_3^- was observed (Fig. 3c) since NaN_3 efficiently

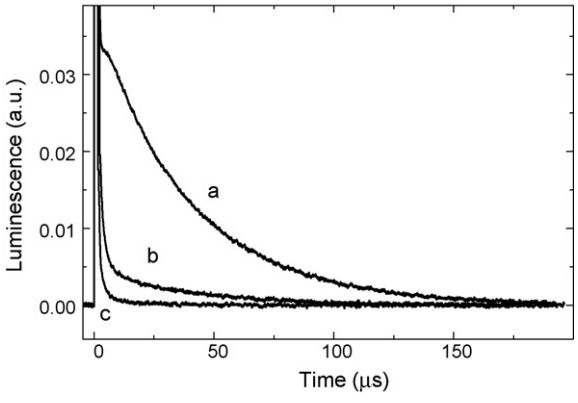

Fig. 2. Time resolved luminescence at 1270 nm in D_2O: 2.6×10^{-5} M TPPS (a), 6×10^{-4} M 2,7-dsDPA (b), 6×10^{-4} M 2,7-dsDPAO$_2$ (c), absorbance at excitation wavelength at 308 nm was 0.156 ± 0.005, laser energy 4.02 mJ/pulse. The slowest monoexponential decay (lifetime~40 μs) can be attributed to $O_2(^1\Delta_g)$ luminescence, the fast process is due to scattering of laser light and emission from the singlet states of photosensitizers.

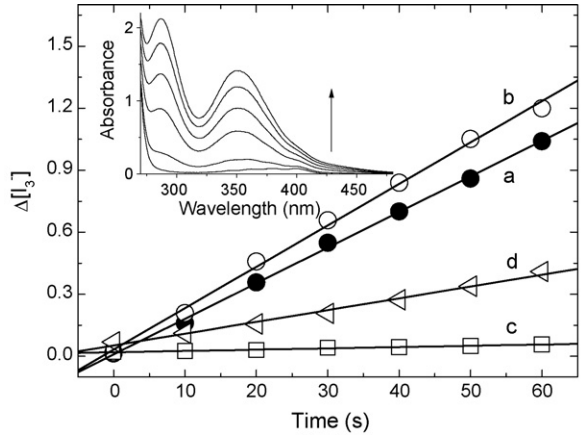

Fig. 3. Photosensitized generation of $O_2(^1\Delta_g)$, measured as I_3^- formation in the time of irradiation. 2,6-dsDPA (1×10^{-5} M) in iodide detection solution (a), the same solution in the presence of D_2O (65%) (b), the same solution in the presence 0.01 M NaN_3 (c) and the same solution in the presence 6.5×10^{-3} M hpγCD (d). Inset: Absorption spectrum of iodide detection solution in the presence of 1×10^{-5} M 2,6-dsDPA after 0, 10, 30, 50, 70 and 90 s of irradiation by a polychromatic 250W halogen lamp. Arrow designates increasing concentration of I_3^-.

quenches $O_2(^1\Delta_g)$ [38,39]. In contrast to bleaching methods using reactions of organic acceptors for detection of O_2 ($^1\Delta_g$), the iodide method has the advantage that it does not use a decrease of the absorbance of an acceptor (followed in bleaching methods), but an increase of the absorbance of the product (I_3^-). This permits the use of acceptor of singlet oxygen (I^-) in large excess. Experiments consisted of monitoring 1270 nm phosphorescence decays of O_2 ($^1\Delta_g$) at various iodide concentrations reveal the rate constant of $8.7 \times 10^5\ M^{-1}\ s^{-1}$ for quenching of O_2 ($^1\Delta_g$) by I^- in aqueous solution [32]. The most important kinetics result is that the I_3^- rise time is the same as the O_2 ($^1\Delta_g$) decay time using 0.1 M concentration of I^- in aqueous solutions [32]. Almost all O_2 ($^1\Delta_g$) generated in 0.1 M I^-, especially in D_2O enriched solutions, yields to I_3^- formation, while solvent quenching and competition with the anthracene moiety (in insignificant concentration to I^-) are negligible.

3.2. Characterization of dsDPAs as $O_2(^1\Delta_g)$ acceptors

The ability of water-soluble 2,6-dsDPA and 2,7-dsDPA to react with $O_2(^1\Delta_g)$ in aqueous solutions was tested using the TPPS photosensitizer with high quantum yield of $O_2(^1\Delta_g)$ [37]. Continuous irradiation of dsDPA air-saturated solutions in the presence of TPPS leads to a remarkable hypochromicity of the absorbance band of dsDPAs at 268 nm (Fig. 4). Similar results were obtained even without the TPPS photosensitizer by using a longer time of irradiation since dsDPA is a poor photosensitizer itself (see Section 3.1).

The endoperoxide formation can be followed also from fluorescence emission spectra. Endoperoxide bond formation results in the dislocation of the conjugate system and thereby in the disappearance of both absorption and emission spectra (Fig. 5).

3.3. Endoperoxides $dsDPAO_2$ as secondary sources of $O_2(^1\Delta_g)$

We did not find any evidence that irradiation of $dsDPAO_2$ by UV light leads to $O_2(^1\Delta_g)$ formation (Fig. 2c), probably due to the absence of an absorption band above 250 nm and pertubation of chromophore aromacity. On the other hand, the reappearance of absorbance band at 268 nm in UV–vis spectrum (Fig. 4) upon heating corresponding to dsDPA formation confirmed thermal decomposition of endoperoxides to parent dsDPAs with 70–80% yield. The thermostability of $dsDPAO_2$ strongly depends on the selected temperature (Fig. 6a, Table 2). The reversible reaction almost did not proceed at low temperature (<60 °C).

To estimate the yield of $O_2(^1\Delta_g)$ released from endoperoxides we designed the following experiment. 1×10^{-5} M 2,7-$dsDPAO_2$ in H_2O and in 0.1 M iodide detection solution in the presence and the absence of 0.01 M NaN_3 was placed in sealed glass ampoules and heated at 125 °C for several minutes. After heating the ampoules were fast cooled to room temperature and the absorbance changes at 268 nm (to detect reappearance of 2,7-dsDPA in H_2O) and at 351 nm (to detect generation of I_3^- in iodide solution) were followed. Assuming that I^- which is in large excess to $O_2(^1\Delta_g)$ quenches singlet oxygen mainly

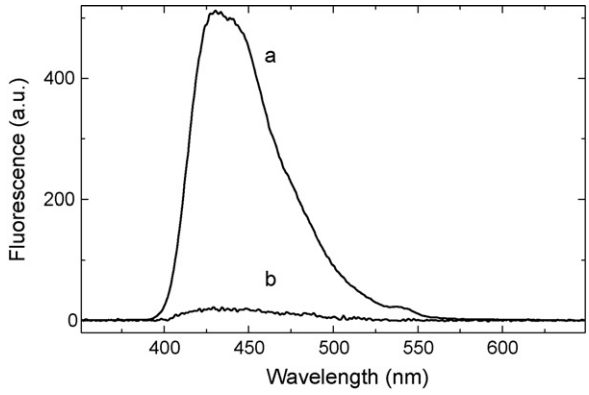

Fig. 5. Fluorescence emission spectra of (a) 2,7-dsDPA (1×10^{-6} M, $\lambda_{exc} = 270$ nm) and (b) corresponding endoperoxide 2,7-$dsDPAO_2$ (2×10^{-5} M, $\lambda_{exc} = 220$ nm) in double-distilled water.

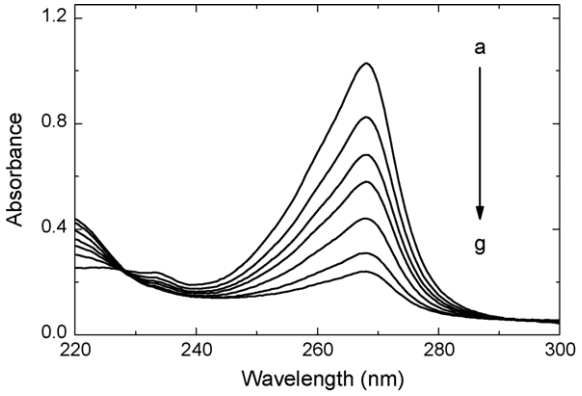

Fig. 4. Absorption spectra of 2,7-dsDPA (1×10^{-5}M) in the presence of TPPS (2×10^{-6}M) in H_2O after 0 min (a), 2 min (b), 4 min (c), 6 min (d), 10 min (e), 18 min (f) and 30 min (g) irradiation by a polychromatic 250 W halogen lamp; the absorption spectrum of TPPS was subtracted for clarity, arrows indicate the decreasing concentration of 2,7-dsDPA due to reaction with $O_2(^1\Delta_g)$.

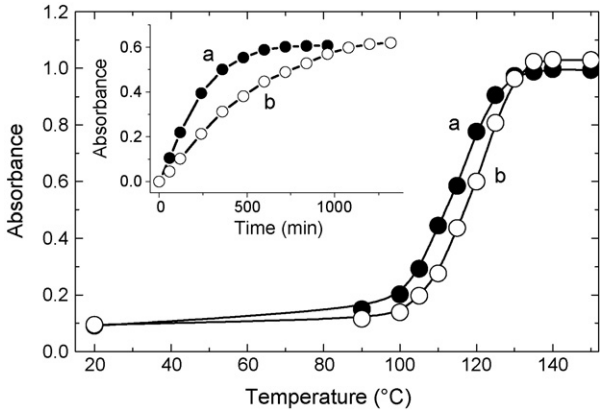

Fig. 6. Thermolysis of endoperoxides to parent compounds. 2×10^{-5} M 2,7-$dsDPAO_2$ in the absence (a) or presence (b) of 8×10^{-3} M hpβCD. Samples were heated in aqueous solutions in closed ampoules. The time of heating was 60 min for all selected temperature. The reappearance of absorbance band of parent compounds was followed at 268 nm (free 2,7-dsDPA) or 271.5 nm (complex with hpβCD). Inset: Comparison of 1×10^{-5} M 2,7-$dsDPAO_2$ thermolysis in the absence (a) and presence (b) of 9×10^{-3} M hpβCD at 100 °C. Samples were in both cases heated in double-distilled water in sealed ampoule.

Table 2
Half lifetimes $t_{1/2}$ for thermolysis of 2,7-dsDPA and 2,7-dsDPA/hpβCD at different temperatures and activation energies E_A calculated from Arrhenius plot

System	E_A (kJ mol^{-1})	$t_{1/2}$ (min)			
		150 °C	125 °C	100 °C	60 °C
2,7-dsDPAO$_2$	73.3 ± 15.3	11.0	18.6	174	2.44×10^4
2,7-dsDPAO$_2$/hpβCD	88.5 ± 16.0	12.7	32.3	360	$>7.50 \times 10^4$

All measurements were performed in double-distilled water in sealed ampoules.

via chemical process (nearly each $O_2(^1\Delta_g)$ oxidize I^- substrate, see 3.1.), and one molecule of $O_2(^1\Delta_g)$ gives two molecules of I_3^- (see Eqs. (2–4)), the yield Φ_Δ can be calculated as:

$$\Phi_\Delta = \left(\frac{[I_3^-]_{abs} - [I_3^-]_{pres}) \times 100}{(2 \times [2,7\text{-dsDPAO}_2] \times r_a} \right), \quad (8)$$

where $[I_3^-]_{abs}$ is the molar concentration of generated I_3^- in iodide solution in the absence of azide, calculated from spectral changes at 351 nm ($\varepsilon = 26{,}450\ M^{-1}\ cm^{-1}$); $[I_3^-]_{pres}$ is the molar concentration of generated I_3^- in the presence of azide quencher, where slightly increased concentration of I_3^- was attributed to enhance oxidation of I^- by $^3O_2$ in air saturated solution at higher temperature; [2,7-dsDPAO$_2$] is the molar concentration of the endoperoxide and r_a is the ratio of 2,7-dsDPA spectral reappearance.

We found 32 ± 3% yield of $O_2(^1\Delta_g)$ released from 2,7-dsDPAO$_2$, which is in good agreement with published data for 9,10-diphenylanthracene endoperoxides [21,28,29].

3.4. Spectral changes, stoichiometry and binding constants of dsDPA/CD complexes

The addition of CDs (hpCDs) to the neutral buffered solutions or deionized double-distilled water of dsDPA results in small changes in absorption and fluorescence spectra. Main bands in absorption and excitation fluorescence spectra are bathochromically shifted from λ = 268 nm up to λ = 271.5 nm (Table 1, Fig. 7). These results are attributed to the formation of a ground state complex between dsDPA and CD and suggest the presence of only two absorbing species in equilibrium in the solutions—the free dsDPA and the dsDPA/CD complex. Formation of complexes is observable also in emission fluorescence spectra, when the presence of CDs results in increasing intensities of emission bands (see Fig. 7 inset). Due to the low dsDPAs concentrations of 1×10^{-5} M, the association takes place between the dsDPAs monomer and a cyclodextrin. In the case of 2,6-dsDPA with βCD, no spectral changes were found.

The stoichiometry of the complexes was assessed by Job's method of continuous variations. For all studied systems (except 2,6-dsDPA/βCD), the maximum of Job plot appears at dsDPA molar fraction of 0.5 (Fig. 8 inset). This indicates a 1:1 stoichiometry of dsDPA/CD complexes (Table 1).

The binding constants K_b of the dsDPA/CD complexes were determined based on binding isotherm analysis (Fig. 8). The best fits to the experimental data confirm that binding in a 1:1 ratio prevails in the studied systems (Table 1).

The values of K_b are dependent on the position of sulfogroups on DPA, the size of the CDs ring and on functionalization of the primary face on CDs. The strongest complexing ability to bind dsDPA was found with hpβCD and hpγCD.

For 2,7-dsDPA, the values of K_b with CDs follow the order: hpβCD > hpγCD > βCD > γCD. The highest K_b values are those with hp(CD, the (-cyclodextrin with the hydroxypropylated primary hydroxyl groups. This modification leads to an order of magnitude increase of binding affinity when compared to native βCD under the same conditions. Since the K_b values are similar for βCD and γCD we can conclude that the proper size of

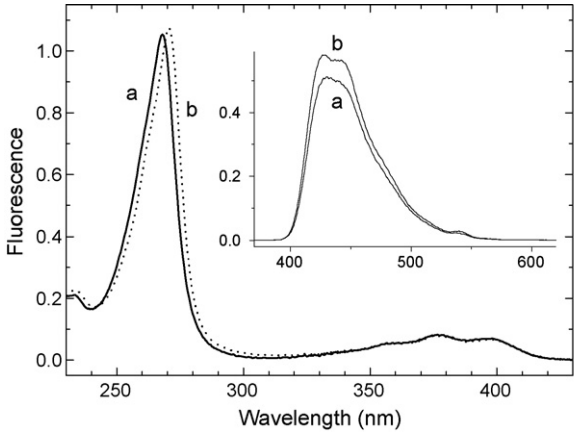

Fig. 7. Fluorescence excitation spectra of (a) 2,7-dsDPA (1×10^{-6} M) and (b) 2,7-dsDPA (1×10^{-6} M)/hpβCD (9×10^{-3} M) complex, emission wavelength λ_{em} = 430 nm. Inset: Emission spectra of the same systems. Excitation wavelength was 270 nm.

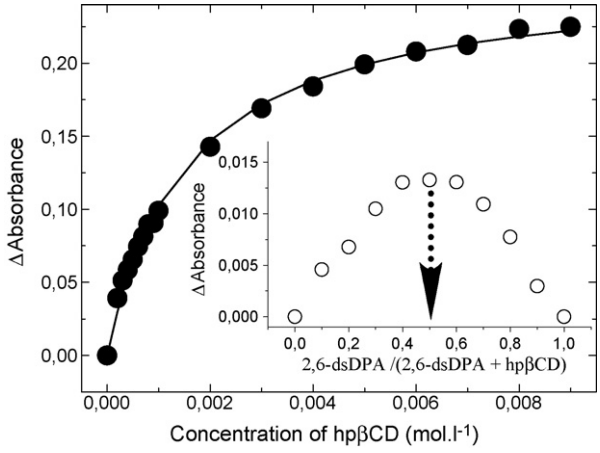

Fig. 8. Binding isotherm for 2,6-dsDPA/hpβCD complex. Inset: Job's plot of the same systems in 0.02 M phosphate buffer, pH 7.0 at 272 nm, total concentration 1×10^{-5} M. The experimental data indicate a 1:1 complex.

Table 3
^{1}H NMR chemical shifts δ_H (ppm) of internal protons H-3, H-5 and external proton H-6,6′ of CDs (see Fig. 1) in the absence or presence of dsDPA

	Protons of CDs		
	H-3	H-5	H-6,6′
δ_H βCD	3.94	3.84	3.86
δ_H 2,7-dsDPA/βCD	**3.82**	**3.78**	3.87
δ_H 2,6-dsDPA/βCD	3.91	3.82	3.86
δ_H 2,7-dsDPAO$_2$/βCD	**3.88**	**3.71**	3.85
δ_H 2,6-dsDPAO$_2$/βCD	3.91	**3.77**	3.86
δ_H γCD	3.94	3.88	3.88
δ_H 2,7-dsDPA/γCD	3.89	**3.78**	**3.81**
δ_H 2,6-dsDPA/γCD	**3.66**	**3.20**	**3.51**
δ_H 2,7-dsDPAO$_2$/γCD	3.93	**3.81**	**3.83**
δ_H 2,6-dsDPAO$_2$/γCD	**3.72**	**3.40**	**3.56**

The bold values indicate the most significance chemical shits.
All samples were measured in D_2O.

the CD molecule is the second important factor for the binding process.

For 2, 6-dsDPA, the values of K_b for complexes with CDs follow a different order: hpγCD > γCD > hpβCD, favoring CDs with a larger cavity. In the case of βCD we did not find any proof of interaction. Again, the presence of 2-hydroxypropyl groups on the primary face of CDs stabilizes the supramolecular complexes. It suggests that the binding site on the dsDPA moiety predominantly interacts with the primary face of the modified cyclodextrins (Fig. 1).

3.5. The mode of dsDPAs/CDs interaction

dsDPA and corresponding endoperoxides dsDPAO$_2$ have similar structures and a similar mode of interaction with CDs can thus be expected. In contrast to dsDPA, interaction of endoperoxides dsDPAO$_2$ with CDs cannot be observed by UV–vis or fluorescence spectroscopy due to the absence of suitable absorption or emission bands.

The presence of dsDPAO$_2$/CD complexes and the modes of interaction of dsDPA/dsDPAO$_2$ with native CDs in aqueous solutions were estimated from ^{1}H NMR measurement. The literature results on NMR are mostly consistent with a model in which some large molecules with peripheral substituents of a proper size and shape are included in the interior of a cyclodextrin cavity [40–43].

The ^{1}H NMR results showed that native CDs form host–guest complexes with all dsDPA/dsDPAO$_2$ except for 2,6-dsDPA/βCD. Differences of chemical shifts of protons located in the cyclodextrin cavity (H-3, H-5 and also H-6 in the case of γCD) and protons of the phenyl groups of dsDPA/dsDPAO$_2$ (H-2″, H-3″ in the case of 2,7-dsDPA/dsDPAO$_2$ and H-2′, H-3′ in the case of 2,6-dsDPA/2,6-dsDPAO$_2$, see Fig. 1) in the absence and presence of CDs complexes were found in ^{1}H NMR spectra (Tables 3 and 4). These results indicate the formation of inclusion complexes, where the phenyl groups of dsDPA/dsDPAO$_2$ are included in the cyclodextrin cavities.

Two binding modes were recognized: (i) Inclusion of dsDPA/dsDPAO$_2$ *via* the secondary face of βCD. The largest chemical shift changes in ^{1}H NMR spectra of βCD complexes were observed for internal proton H-3 and H-5. Differences in chemical shifts of external protons H-6,6′ located on the primary face were not found (Table 3). It indicates the formation of inclusion complexes with a binding mode *via* the secondary face. (ii) Inclusion of dsDPA/dsDPAO$_2$ *via* the primary face of γCD. The size of the secondary face of γCD is broader (cavity diameter: 7.5–8.3 Å) than of βCD (cavity diameter: 6.0–6.5 Å), and resembles the primary face of βCD; the inclusion could therefore be more favorable from the primary face. This presumption was confirmed by the chemical shift changes of external protons H-6,6′ on the primary face and internal protons H-5 (Table 3). Table 4 also indicates that complexes were formed *via* insertion of phenyl groups of dsDPA/dsDPAO$_2$ into the cyclodextrin cavities. In the case of 2,7-dsDPA/2,7-dsDPAO$_2$, the contacts were attributed to phenyl groups more distant from the sulfonated part of anthracene molecules (see Fig. 1).

3.6. Influence of CDs on dsDPAs and dsDPAO$_2$s properties

The influence of CDs on the photosensitized formation $O_2(^1\Delta_g)$ from dsDPA was also studied. The supramolecular host–guest complexes with the highest K_b (2,7-dsDPA/hpβCD and 2,6-dsDPA/hpγCD) have been chosen for this observation. A lower production of $O_2(^1\Delta_g)$ was found in the presence CDs (Fig. 3d), probably due to the lower diffusion rate of O_2 to the excited molecules of dsDPAs in cyclodextrin solutions, as was reported for similar systems [16].

The presence of CDs slightly slowed down the time course of dsDPAs photobleaching (Fig. 9). It can be attributed to a combination of several factors, e.g. complexation of dsDPAs by CDs, lowering of the rate of oxidation due to steric effects and the fact that cyclodextrins are weak deactivating agents of singlet oxygen with the upper limits for the bimolecular deactivation constants of 2×10^5 and 1×10^5 M^{-1} s^{-1} for βCD

Table 4
^{1}H NMR chemical shifts δ_H (ppm) of the phenyl group of anthracene derivatives and in the absence and the presence of native CDs

	2,7-dsDPA		2,6-dsDPA		2,7-dsDPAO$_2$		2,6-dsDPAO$_2$	
	H-2″	H-3″	H-2′	H-3′	H-2″	H-3″	H-2′	H-3′
δ_H guest	7.43	7.65	7.39	7.69	7.61	7.58	7.72	7.72
δ_H guest/βCD	**7.47**	7.67	**7.49**	7.71	**7.74**	**7.78**	**7.76**	**7.76**
δ_H guest/γCD	7.44	7.66	**7.48**	**7.64**	**7.72**	**7.79**	**7.76**	**7.76**

The bold values indicate the most significance chemical shits.
All samples were measured in D_2O.

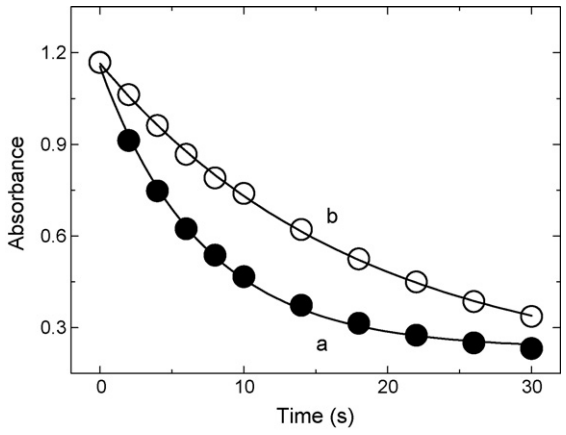

Fig. 9. The time dependence of photosensitized formation of 2,6-dsDPAO$_2$ in the presence (a) and absence (b) of hpγCD. The initial concentrations of 2,6-dsDPA and hpγCD were 1×10^{-5} M and 8×10^{-3} M, respectively, TPPS (2×10^{-6}M) was used as a photosensitizer. The absorbance of free 2,6-dsDPA was followed at 268 nm, the absorbance of its complex with hpγCD was followed at 270.5 nm. The irradiation was carried out by a polychromatic 250 W lamp in double-distilled water.

and hpβCD, respectively [44], i.e. 2–3 orders less than value typical for water-soluble anthracene derivates such as 9,10-anthracenedipropionic acid with bimolecular deactivation rate constant of 8.2×10^7 M^{-1} s^{-1} [45].

The influence of CD on thermostability of dsDPAO$_2$s was examined for 2,7-dsDPAO$_2$ in the absence and presence of hpβCD that forms a complex with the highest K_b with 2,7-dsDPA (Table 1). The activation energies calculated from Arrhenius equation yield 73.3 ± 15.3 and 88.5 ± 16.0 kJ mol^{-1} for 2,7-dsDPAO$_2$ and 2,7-dsDPAO$_2$ in complex with hpβCD, respectively (Table 2). The ability of cyclodextrins to increase the endoperoxide thermostability was found at lower temperatures, as illustrated by the half-times of decomposition ($t_{1/2}$) calculated for all tested temperatures (Table 2).

4. Conclusion

We found that dsDPA represent water-soluble photosensitizers and acceptors of $O_2(^1\Delta_g)$ forming thermostable endoperoxides. dsDPA and corresponding endoperoxides dsDPAO$_2$ form 1:1 supramolecular complexes with CDs and hpCDs in neutral aqueous solutions. Two binding modes were recognized on the basis of NMR experiments. Inclusion of dsDPA/dsDPAO$_2$ *via* the secondary face of CDs is a typical mode for 2,6-dsDPAO$_2$ or 2,7-dsDPA/2,7-dsDPAO$_2$ interaction with βCD. Inclusion *via* the primary face was found for dsDPAs or dsDPAO$_2$s complexes with γCD. A non-covalent interaction between the dsDPAs and CDs/hpCDs affects their photophysical and physical properties such as the bathochromic shift of the absorption band of dsDPA, decreasing photoproduction of $O_2(^1\Delta_g)$ and oxidation rate of dsDPA by $O_2(^1\Delta_g)$, and a remarkable increase in the thermal stability of dsDPAO$_2$ endoperoxides.

In conclusion, water-soluble dsDPAs can serve as poor photosensitizers, excellent $O_2(^1\Delta_g)$ acceptors forming thermostable water-soluble endoperoxides, and guest molecules for host–guest interactions with CDs. Endoperoxides dsDPAO$_2$s bound to CD cavity can be considered as extremely thermostable supramolecular secondary sources or carriers of $O_2(^1\Delta_g)$, utilized for several mechanistic $O_2(^1\Delta_g)$ studies and organic synthesis.

Acknowledgement

This work was supported by the Czech Science Foundation (grants 203/07/1424 and 203/06/1244).

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