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Singlet oxygen phosphorescence photosensitized in nano-aggregates of C₆₀ buckminsterfullerene is insensitive to solvent and quenchers and strongly red-shifted indicating highly polarizable interior

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Abstract

We investigated the strongly red-shifted singlet oxygen (¹O₂) phosphorescence spectra in an aqueous preparation of C₆₀ buckminsterfullerene. The ~10 nm red shift was associated with H₂O dispersions of C₆₀ nanoaggregates (C₆₀)_n that can photosensitize ¹O₂ in their polarizable cores. In contrast to ¹O₂ produced by the water-soluble C₆₀-(γ-cyclodextrin)₂ complex, ¹O₂ trapped inside (C₆₀)_n was short-lived (~2–3 μs), insensitive to solvent and ¹O₂ quenchers, and did not induce photocytotoxicity. To our knowledge, ¹O₂ spectrum from (C₆₀)_n is the most red-shifted ¹O₂ spectrum recorded to date and it may be used to probe the inner polarizability of carbon (nano)aggregates.

Introduction

Buckminsterfullerene C₆₀ is a known photosensitizer of singlet oxygen (¹O₂) in nonpolar solvents (1,2). Much effort has been expended to solubilize C₆₀ in H₂O. One successful strategy is to disperse C₆₀ by different means (3,4) to form nano-aggregates (C₆₀)_n. Solubilization of C₆₀ in aqueous media is driven by biological applications that may involve photosensitization of ¹O₂. The latter is best studied spectrally by its IR phosphorescence. However, we did not observe any correlation between the spectrally determined ¹O₂ production by different aqueous preparations of C₆₀ and their *in vitro* phototoxicities. Interestingly, we noticed that the spectra of ¹O₂ were often red-shifted in aqueous (C₆₀)_n dispersions which prompted us to examine ¹O₂ production by aggregated C₆₀ in more detail.

Experimental Section

Singlet oxygen lifetimes were measured using a laser pulse spectrometer described in detail elsewhere (6). Briefly, the apparatus utilized a Surelite II laser (Continuum, Santa Clara, CA) for excitation. A germanium diode (Model 403 HS, Applied Detector Corporation, Fresno, California) in conjunction with an efficient optical system was used for signal detection. Data were acquired on a HP 54111D Digitizing Oscilloscope (Hewlett Packard Colorado Springs, CO) interfaced to a PC computer. Singlet oxygen was produced by single

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pulse excitation of C_{60} preparations at 355 nm (5 ns duration and ca. 35 mJ energy). The 1O_2 lifetime was calculated from the monoexponential decay of its phosphorescence observed through an interference filter at 1270 nm.

The steady-state 1O_2 phosphorescence spectra were recorded on a steady-state 1O_2 spectrophotometer featuring an optimized optical system as in our pulse 1O_2 spectrophotometer. Samples were excited from a 500 Watts Hg lamp operating at 300 Watts through a 366 nm interference filter in combination with KG3 heat-removing glass filter. The 1O_2 phosphorescence spectra were recorded over the range of 1200–1350nm during one ca. 30 s scan and were normalized to the same number of absorbed photons at the excitation wavelength. Perinaphthenone was used as a standard for the 1O_2 quantum yield calculations.

Absorption spectra were measured using a HP Diode Array Spectrophotometer model 8452A (Hewlett Packard Co., Palo Alto, CA). The relative number of absorbed photons at the excitation wavelength was calculated using the Beer-Lambert law. The samples were prepared in a suprasil fluorescence cuvette (0.5 cm pathlength), and were air-equilibrated or purged with either oxygen or nitrogen gasses, as required.

Transmission electron microscope (TEM) images were taken on a Tecnai-12 Bio-Twin transmission electron microscope (FEI, The Netherlands.) operating at 80 kV. TEM grids were prepared by evaporating approximately 20 μ L of aqueous fullerenes solution/dispersion onto a 300 mesh carbon-coated copper grid.

Results and Discussion

The steady-state spectrum of 1O_2 phosphorescence measured from $(C_{60})_n$ in O_2 -saturated D_2O is shown in Fig. 1, while Fig. 2 shows the corresponding absorption spectrum; the transmission electron microscope (TEM) image of $(C_{60})_n$ nanoparticles is shown in the insert. Fig.1 also shows 1O_2 emission spectrum generated by C_{60} in toluene. The peak position of this spectrum is 1273 nm, while the 1O_2 spectrum from $(C_{60})_n$ in D_2O is red-shifted by ca. 10 nm. A similar red shift was consistently observed for $(C_{60})_n$ dispersed without any additives in H_2O . The red-shifted spectrum in D_2O or H_2O is due to 1O_2 , because it was totally dependent on the concentration of oxygen (5) (Fig.3). Results from time-resolved technique (6) support this conclusion.

Surprisingly, the 1O_2 lifetime (τ_{so}) photosensitized by the $(C_{60})_n$ dispersion in D_2O was the same as that in H_2O , even though τ_{so} is ca. 50 μ s in our D_2O sample (7), compared to 3.1 μ s in water (8). Fig. 4 shows the decays of 1O_2 produced by $(C_{60})_n$ and $C_{60}-(\gamma-CD)_2$ in D_2O , and by C_{60} in toluene. The 1O_2 transient decay of $(C_{60})_n$ dispersed in H_2O then extracted into toluene is also presented. The decay of 1O_2 produced by $C_{60}-(\gamma-CD)_2$ confirms that τ_{so} in our D_2O sample is $\sim 50 \mu$ s. A lack of τ_{so} increase in $(C_{60})_n/D_2O$ suggests that 1O_2 emission was not from bulk D_2O , but rather from the $(C_{60})_n$ core (9). To test this assumption we applied two efficient physical 1O_2 quenchers: NaN_3 in H_2O/D_2O (10), and strychnine (11) in toluene. We also used a chemical quencher 2,5-dimethylfuran, which was effective in solution but not in the nanoaggregates (not shown).

Quenchers provide information on $^1\text{O}_2$ fate in solution. However, if the quenchers are too large to penetrate the aggregates, they will not quench $^1\text{O}_2$ there. The cavities in $(\text{C}_{60})_n$ seem to be small and penetrable only by dissolved O_2 , which facilitates $^1\text{O}_2$ production. We found that, indeed, whenever $^1\text{O}_2$ spectrum was red-shifted, it was largely unaffected by quenchers present at concentrations that totally abolish the usual $^1\text{O}_2$ emission. These observations support our notion that $^1\text{O}_2$ must be photoproduced and then deactivated mostly inside the $(\text{C}_{60})_n$ aggregates and not in solution. The τ_{so} of $^1\text{O}_2$ inside the $(\text{C}_{60})_n$ is solvent-independent; its estimated value, ca. 3 μs , is close to the τ_{so} in H_2O . It seems that the conjugated double bond system of C_{60} quenches $^1\text{O}_2$, thus controlling its lifetime in the $(\text{C}_{60})_n$ microenvironment. The π electrons are also responsible for the high polarizability of the C_{60} aggregates (*vide infra*). These results show that two milieus must be considered for proper analysis of $^1\text{O}_2$ photosensitization by aggregated C_{60} in aqueous solutions. We modeled this by using C_{60} and γ -cyclodextrin (γ -CD).

We prepared non-aggregated water-soluble C_{60} by complexation with γ -CD (12,13). The $^1\text{O}_2$ spectrum photogenerated by the C_{60} - $(\gamma\text{-CD})_2$ complex in D_2O does not appear to be red shifted and is sensitive to $^1\text{O}_2$ quenchers (Fig. 5). However, upon total quenching of the emission from D_2O , there was a small contribution from the red-shifted $^1\text{O}_2$ spectrum, revealing the presence of $(\text{C}_{60})_n$ (Fig. 5). Relative contribution from the $(\text{C}_{60})_n$ emission was even higher for $\text{H}_2\text{O}/\gamma\text{-CD}$ (Fig.5) due to the shorter $\tau_{\text{so}}(\text{H}_2\text{O})$ diminishing the usual $^1\text{O}_2$ emission more in H_2O than in D_2O . C_{60} - $(\gamma\text{-CD})_2$ is not stable in H_2O forming yellow colored $(\text{C}_{60})_n$ over time, a process that is accelerated by heat. Thus, both soluble monomeric C_{60} and dispersed $(\text{C}_{60})_n$ nanoparticles can be present simultaneously in aqueous solutions containing γ -CD.

The existence of two clearly different milieus may affect calculation of $^1\text{O}_2$ quantum yield (Φ_{so}) based on spectral data. Φ_{so} is usually measured against a known standard applied in the same solvent, which ensures the same nonradiative ($1/\tau_{\text{so}}$) and radiative (k_r) rate constants for $^1\text{O}_2$ decay. While both constants are solvent-dependent (14–17), k_r is more difficult to measure and compensation between different environments is more complicated due to lack of reliable values. Obviously, simple comparison of $^1\text{O}_2$ spectra may yield incorrect Φ_{so} , if more than a single milieu is involved in $^1\text{O}_2$ processes. We measured Φ_{so} by different C_{60} preparations (Table 1) considering two milieus, when pertinent.

Our measurements for C_{60} in solution are in general agreement with the published Φ_{so} values compiled by Prat *et al.* (18) who also investigated the inclusion complexes of C_{60} with γ -cyclodextrin. These complexes featured close to unity triplet quantum yields and high Φ_{so} values (18). For C_{60} nanoaggregates, the accuracy of our ϕ estimation in the aggregates is determined primarily by the unknown radiative rate constant. This constant cannot be eliminated (reduced) from the equation by the usual application of $^1\text{O}_2$ standard, as there is no $^1\text{O}_2$ reference standard small enough to be placed into the cavity of C_{60} aggregates. Therefore, our ϕ estimate (Table 1) is based on extrapolation from the published polarizability dependence (16). While this arbitrary procedure can produce ϕ values larger than unity, it does suggest that ϕ is close to unity in oxygen purged solutions when the aggregate cavities are saturated with oxygen molecules.

Notice that physicochemically correct Φ_{so} values may not always correlate with $^1\text{O}_2$ -induced photocytotoxicity. A simple correlation is expected only for $^1\text{O}_2$ production in solution, but not inside the $(\text{C}_{60})_n$ particles that deactivate $^1\text{O}_2$ prior to diffusion out. Thus, only $\text{C}_{60}-(\gamma\text{-CD})_2$, which is a perfect $^1\text{O}_2$ generator (Table 1), was phototoxic to cell cultures, while $(\text{C}_{60})_n$ was not (to be published later). A correct understanding of $^1\text{O}_2$ generation by aqueous C_{60} preparations allows us to reliably predict phototoxicity by considering $^1\text{O}_2$ sensitivity to solvent and quenchers, which concurs with the spectral shift of $^1\text{O}_2$ emission.

What is the origin of this red shift? Unlike k_r and τ_{so} , $^1\text{O}_2$ emission energy is less affected by solvents. Nonetheless, red shifts have been observed (19–21) and linked to increasing solvent polarizability (α) (16). The most red-shifted $^1\text{O}_2$ spectra were in iodinated solvents (16), reaching a maximum at around 7824 cm^{-1} (1278 nm). The $^1\text{O}_2$ spectrum that we measured in methylene iodide ($n=1.74$) shows similarly red-shifted ($\sim 9\text{ nm}$) peak position (not shown). The spectrum in $(\text{C}_{60})_n$ is even more red-shifted (7798 cm^{-1}), extrapolating (16) to $\alpha \approx 0.46$ that would match solvent refractive index $n=1.887$ (in diamond $n=2.417$ (22)). Moreover, the red shifts were empirically associated with the increasing k_r values (16), hence, k_r should also be enhanced in $(\text{C}_{60})_n$. We used this association to estimate k_r inside $(\text{C}_{60})_n$ for $^1\text{O}_2$ quantum yield calculation shown in Table 1.

The above α estimate reveals a very polarizable $(\text{C}_{60})_n$ core probed by $^1\text{O}_2$. This core is hydrophobic and not accessible to solvent but to O_2 that slowly diffuses in and out with a dwelling time longer than $3\text{ }\mu\text{s}$ ($^1\text{O}_2$ cannot diffuse out in its lifetime). While at least four C_{60} must aggregate to create a protective cavity for O_2 , the average $(\text{C}_{60})_n$ size in our preparation estimated from the TEM images was $n=30\text{--}60$ molecules. $(\text{C}_{60})_n$ seems to be a dynamic formation showing Brownian motion that creates cavities large enough (23) to host O_2 that, upon excitation, can convert to $^1\text{O}_2$ (Fig. 5).

In summary, to our knowledge, we found the most red-shifted $^1\text{O}_2$ phosphorescence spectrum recorded to date generated in aqueous preparations of $(\text{C}_{60})_n$. The $^1\text{O}_2$ spectral shift concurs with $^1\text{O}_2$ sensitivity to solvent and quenchers, which may be used to reliably predict phototoxicity exerted by $^1\text{O}_2$ in different preparations of carbon nanoaggregates. Moreover, since the $^1\text{O}_2$ spectral red shift correlates well with polarizability, $^1\text{O}_2$ acts as a probe providing a unique insight into the inner polarizability of $(\text{C}_{60})_n$ aggregates.

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Figure 1.

$^1\text{O}_2$ emission spectra from C_{60} in toluene and $(\text{C}_{60})_n$ in D_2O in the absence and presence of strychnine (toluene) and NaN_3 (D_2O). While NaN_3 at 2 mM did not show any $^1\text{O}_2$ quenching (not shown), the $^1\text{O}_2$ quenching by NaN_3 at 100 mM may also involve C_{60} excited state(s). Spectra in toluene were reduced ca. 4 times; $\lambda_{\text{ex}}=366$ nm.

Figure 2. Absorption spectra of C₆₀ in toluene and C₆₀-(γ-CD)₂ and (C₆₀)_n in D₂O. Insert shows TEM image of (C₆₀)_n.

Figure 3.
 $^1\text{O}_2$ emission spectra from $(\text{C}_{60})_n$ in D_2O saturated with oxygen, air and nitrogen.

Figure 4.

Decays of $^1\text{O}_2$ emission photosensitized by $(\text{C}_{60})_n$ and $\text{C}_{60}-(\gamma\text{-CD})_2$ in D_2O and by C_{60} in toluene. Back extraction of $(\text{C}_{60})_n$ from D_2O to toluene (adjacent curve) produced the same $\tau_{\text{so}} = 28.7 \mu\text{s}$. Decays were produced by single laser pulses 355 nm.

Figure 5.

$^1\text{O}_2$ emission spectra from $\text{C}_{60}-(\gamma\text{-CD})_2$ in D_2O and H_2O in the absence and presence of NaN_3 (D_2O). As expected, the spectrum photosensitized by perinaphthenone (P) was completely quenchable by azide; NaN_3 (2 mM) also totally quenched “the usual” $^1\text{O}_2$ emission from $\text{C}_{60}-(\gamma\text{-CD})_2$ (not shown). $\lambda_{\text{ex}}=366$ nm.

Table 1

The quantum yield of $^1\text{O}_2$ photosensitization (Φ_{so}) by different C_{60} preparations.

C_{60} Preparation	Φ_{so}	$\Phi_{\text{so}}^{\text{solution}}$	Phototoxicity
$\text{C}_{60}/\text{toluene}$	1	1	N/A
$(\text{C}_{60})_{\text{n}}/\text{D}_2\text{O}/\text{H}_2\text{O}$	$\sim 1^*$	0	No
$\text{C}_{60}-(\gamma\text{-CD})_2/\text{D}_2\text{O}$	1	1	Yes

Measurements used perinaphthenone as a $^1\text{O}_2$ photosensitization standard (24).

* For calculations in $(\text{C}_{60})_{\text{n}}/\text{D}_2\text{O}$, k_{r} was estimated from an extrapolation based on the α dependence (16), while $1/\tau_{\text{so}}$ was measured inside $(\text{C}_{60})_{\text{n}}$ and in solutions. The following values were used: k_{r} , 0.21, τ_{so} 54 μs in D_2O , and k_{r} , 3.8, τ_{so} 2.9 μs in $(\text{C}_{60})_{\text{n}}$. All solutions were saturated with oxygen, and $\lambda=366$ nm was used for excitation. It is obvious that Φ_{so} calculations for different $(\text{C}_{60})_{\text{n}}$ preparations could produce a range of nonsense values (not shown), if a single milieu would had been considered. N/A, not applicable.