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Singlet oxygen generation from Li\textsuperscript{+}@C\textsubscript{60} nano-aggregates dispersed by laser irradiation in aqueous solution\textsuperscript{+}

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Laser pulse irradiation of a deaerated aqueous solution containing solid state of lithium ion-encapsulated fullerene resulted in formation of highly dispersed nano-aggregates [(Li\textsuperscript{+}@C\textsubscript{60})\textsubscript{n}]. Photoirradiation of an O\textsubscript{2}-saturated D\textsubscript{2}O solution containing [(Li\textsuperscript{+}@C\textsubscript{60})\textsubscript{n}] gave singlet oxygen with 55\% quantum yield, leading to the efficient double-stranded DNA cleavage.

Photodynamic therapy (PDT) has developed as a non-invasive clinical treatment of various dermatological, ophthalmic and cardiovascular diseases.\textsuperscript{1-6} The tumour cell apoptosis in the PDT treatment is carried out by photoirradiation of photosensitiser to generate the reactive oxygen species (ROS) such as singlet oxygen (O\textsuperscript{1}g) and superoxide (O\textsuperscript{2}–) in the malignant tumour.\textsuperscript{1-6} The requirements of an ideal photosensitiser are water solubility, low cytotoxicity in the dark, high stability against light, high tumour specificity, high ability to produce ROS and rapid metabolism.\textsuperscript{1,6}

Fullerenes, especially [60]fullerene (C\textsubscript{60}), are known as efficient photosensitisers to generate the triplet excited state and ROS with high quantum yields (\(\Phi(T) = 0.98\); \(\Phi(T) = 0.96\) in C\textsubscript{60}D\textsubscript{30}).\textsuperscript{7} Additionally, fullerenes are remarkably photostable and non-toxic reagents.\textsuperscript{8} However, pristine C\textsubscript{60} is hardly soluble in water (0.4 \(\mu\)g mL\textsuperscript{-1} at 298 K)\textsuperscript{9} and biological media to prevent expression of the photoactivity and PDT efficiency.\textsuperscript{6} Therefore, various fullerene derivatives, such as C\textsubscript{60} with polyethylene glycol,\textsuperscript{10} and \(\gamma\)-cyclodextrin,\textsuperscript{11-13} liposome,\textsuperscript{14} and porous silicon-incorporated C\textsubscript{60}\textsuperscript{15,16} have been reported to improve the water solubility.\textsuperscript{16,17} Introductions of water-soluble substituents have also been reported, however, molecular C\textsubscript{60} and substituted C\textsubscript{60} have no strong absorption around 600-800 nm. Fullerene dispersion suspended in water is also reported by the reprecipitation, solvent replacement, ultrasonication and laser ablation methods.\textsuperscript{18-22}

Recently, a lithium ion-encapsulated fullerene hexafluorophosphate salt (Li\textsuperscript{+}@C\textsubscript{60}PF\textsubscript{6}–) has been reported as an efficient photosensitiser to form the long-lived triplet excited state, which is comparable to that of C\textsubscript{60}.\textsuperscript{23} However, neither solubilisation of Li\textsuperscript{+}@C\textsubscript{60} or C\textsubscript{70} to water nor the photoinduced singlet oxygen generation efficiency has been studied. We report herein highly water-dispersed heterogeneous fullerene nano-aggregates composed of Li\textsuperscript{+}@C\textsubscript{60}, C\textsubscript{60}, and C\textsubscript{70}, which have absorption bands in the visible region as well as an efficient singlet oxygen generation properties.

The solubility of Li\textsuperscript{+}@C\textsubscript{60}PF\textsubscript{6}– salt is extremely low in water as shown in the inset pictures in Fig. 1a, where the black powders are deposited in the bottom of the cuvette. Laser pulse irradiation (\(\lambda = 532\) nm; 500 mW; 10 Hz, 60 min, i.d. = 8 mm) of a deaerated aqueous solution (2.5 mL) containing the dispersed Li\textsuperscript{+}@C\textsubscript{60}PF\textsubscript{6}– salt (1.0 mg) resulted in formation of Li\textsuperscript{+}@C\textsubscript{60} nano-aggregates [(Li\textsuperscript{+}@C\textsubscript{60})\textsubscript{n}]. A brown colour supernatant solution containing nano-aggregates was obtained.

![Fig. 1](image-url)
after the centrifugation (15,000 rpm for 10 min) and the
decantation procedures. The UV-vis absorption spectra of nano-
aggregates in water are shown in Fig. 1, exhibiting two
characteristic absorption bands for (Li$^+$@C$_{60}$)$_n$ in water at 264
and 340 nm which are red-shifted as compared in a
dichloromethane solution (257 and 327 nm) by aggregation. A
broad shoulder absorption band is also shown around 400-600
nm, which is characteristic of an intermolecular charge-transfer
(CT) transition between fullerenes in the nano-aggregates. A
similar CT band was observed for (C$_{60}$)$_n$. Such a CT band has
been reported in the case of a C$_{60}$ thin film. The enhancement
of solubility of (Li$^+$@C$_{60}$)$_n$ and (C$_{60}$)$_n$ in water may be obtained
by CT interactions. The solubilisation of C$_{60}$ aggregates may
occur without the substitution and decomposition to the
fullerene cages, which was confirmed by MALDI-TOF-MS
spectral measurements indicating the only peak due to non-
substituted fullerene.

The dynamic light scattering (DLS) measurements were
performed to evaluate the size of (Li$^+$@C$_{60}$)$_n$ as shown in Fig.
2. The size of the nano-aggregates was significantly decreased
to 30 nm by the laser pulse excitations. Thus, one nano-
aggregate consists of ca. 30,000 Li$^+$@C$_{60}$ molecules. When Li$^+$@C$_{60}$PF$_6$ was replaced by C$_{60}$ and C$_{70}$, the highly dispersed
nano-aggregates were also obtained by the laser pulse
irradiation. The sizes of C$_{60}$ and C$_{70}$ nano-aggregates were
estimated to be 52 and 64 nm, which are larger than
(Li$^+$@C$_{60}$)$_n$. Transmission electron microscopy (TEM)
measurements of (Li$^+$@C$_{60}$)$_n$ were performed to evaluate the
formation of nano-aggregates, indicating the grape bunch
morphology of the nano-aggregates of 30-40 nm in size (Fig.
3). The (Li$^+$@C$_{60}$)$_n$ solution was stable at room temperature for
three days without re-aggregation.

Photoirradiation of an oxygen-saturated deuterated water
(D$_2$O) solution of (Li$^+$@C$_{60}$)$_n$ results in formation of singlet
oxygen, which was detected by the $^1$O$_2$ phosphorescence at
1270 nm (Fig. 4). The quantum yields ($\Phi$) of $^1$O$_2$ generation
were determined from the phosphorescence intensity, which
was compared to the intensity obtained using rose bengal as a
reference compound ($\Phi$ = 0.77). Relatively high $\Phi$ values are
obtained and the values are summarised in Table 1, in which
the highest $\Phi$ value is 0.55 for (Li$^+$@C$_{60}$)$_n$. The values of nano-
aggregates are smaller than those of the corresponding
fullerenes in C$_{60}$/C$_{60}$H$_{12}$CN (1:1 v/v) probably because of the
excited state annihilation (vide infra).

Femtosecond and nanosecond time-resolved transient
absorption spectral measurements were performed to clarify the
excited state dynamics and reaction mechanisms for the
formation of singlet oxygen from (Li$^+$@C$_{60}$)$_n$. Ultrashort
photodynamics for the intersystem crossing (ISC) from the
singlet to the triplet excited state of (Li$^+$@C$_{60}$)$_n$ was observed by
femtosecond laser flash photolysis. The transient absorption
band at 960 nm taken at 10 ps after the femtosecond laser pulse
excitation at 393 nm is assigned to the singlet excited state of
Li$^+$@C$_{60}$ [1(Li$^+$@C$_{60}$)] in nano-aggregates (Fig. 5), which is
relatively broadened as compared to the singlet-singlet
absorption of Li$^+$@C$_{60}$ in PhCN. The decay of absorbance at

Fig. 2 Particles size distributions determined by dynamic light scattering (DLS) of
(a) (Li$^+$@C$_{60}$)$_n$, (b) C$_{60}$, and (c) C$_{70}$.

Fig. 3 TEM images of (Li$^+$@C$_{60}$)$_n$. (a) Large scale and (b) detailed views.

Fig. 4 Emission spectra of $^1$O$_2$ obtained by photoirradiation (\(\lambda = 532\) nm) of O$_2$-saturated
D$_2$O solutions containing [Li$^+$@C$_{60}$], [C$_{60}$], and [C$_{70}$], in at 298 K.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\Phi^a$</th>
<th>Sample</th>
<th>$\Phi^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li$^+$@C$_{60}$</td>
<td>0.83</td>
<td>(Li$^+$@C$_{60}$)$_n$</td>
<td>0.55</td>
</tr>
<tr>
<td>C$_{60}$</td>
<td>0.96$^c$</td>
<td>(C$_{60}$)$_n$</td>
<td>0.30</td>
</tr>
<tr>
<td>C$_{70}$</td>
<td>0.81$^c$</td>
<td>(C$_{70}$)$_n$</td>
<td>0.41</td>
</tr>
</tbody>
</table>

$^a$ Dissolved in C$_{60}$/H$_2$CN (1:1 v/v). Emission data are shown in Fig. S1 in
ESI. $^b$ Dispersed in D$_2$O. $^c$ Taken from ref 6.
900 nm obeyed two-exponential curve. The faster component could be assigned to the singlet-singlet annihilation in the (Li+@C_{60})_n nano-aggregates because the ratio of faster component increased with increasing the excitation laser power intensity without changing the rate constant \( (3.8 \times 10^{12} \text{ s}^{-1}) \) (Fig. S2 in the electronic supplementary information (ESI) †). The residual absorption band at 900 nm was slowly decayed with appearance of the absorption band at 700 nm due to the triplet exited state of Li+@C_{60} dimer.\(^{26}\) The decay rate constant of the slower part was determined to be \( 6.6 \times 10^8 \text{ s}^{-1} \), which is virtually the same as that of formation of the triplet excited state of Li+@C_{60} (7.0 \times 10^8 \text{ s}^{-1}) (see Fig. S3 in ESI †). This value is slightly smaller than the value of homogeneous Li+@C_{60} in PhCN (8.9 \times 10^8 \text{ s}^{-1}).\(^{27}\)

The triplet exited state of (Li+@C_{60})_n is also detected by the transient absorption spectral measurements observed in a strictly deaerated aqueous solution after nanosecond laser excitation at 355 nm. The transient absorption band taken at 20 ns are due to the triplet-triplet (T-T) transition (see Fig. S3a in ESI †). The band is significantly broadened compared to the case of Li+@C_{60} in PhCN. The T-T absorption maximum of (Li+@C_{60})_n is virtually the same as that of Li+@C_{60} (\( \lambda_{\text{max}} = 750 \text{ nm} \)).\(^{27}\) This suggests the aggregation with strong \( \pi \) stacking between the fullerene cages in (Li+@C_{60}). The decay of T-T absorption obeyed the first-order kinetics. The lifetime of the transient species was determined to be 32 ns (Fig. S4 in ESI †). There was no contribution of the T-T annihilation, because the triplet lifetime remained constant at different laser power intensities (Fig. S4b in ESI †). The short triplet lifetime may result from the strong \( \pi \) stacking between the fullerene cages in (Li+@C_{60})_n. On the other hand, no T-T absorption spectrum was observed when (Li+@C_{60})_n was replaced by (C_{60})_n and (C_{60}) with under similar experimental conditions.\(^{28}\) The \( \pi \) stacking in (Li+@C_{60})_n is much weaker than those of (C_{60})_n and (C_{60})_n, because (Li+@C_{60})_n contains some PF_6^- counter anions in the nano-aggregates to avoid \( \pi-\pi \) interaction between the fullerene cages.\(^{29}\)

The triplet exited state of (Li+@C_{60})_n can be an active species for formation of singlet oxygen by energy transfer with molecular O_2. We also examined the DNA-cleave activity of (Li+@C_{60})_n in the presence of O_2 using the widely used assay with the supercoiled double-stranded plasmid DNA, pBR322, because singlet oxygen is formed by the photoirradiation of (Li+@C_{60})_n in an aqueous solution. The agarose gel electrophoresis was performed after 10 h photoirradiation of pBR 322 with a xenon lamp (\( \lambda > 380 \text{ nm} \)) in the presence of (Li+@C_{60})_n in comparison with the control experiments as shown in Fig. 6a. Photoirradiation of (Li+@C_{60})_n in the presence of O_2 is significantly effective for DNA cleavage due to the singlet oxygen generation due to the observation of large amount of cleaved DNA (Form II). The DNA cleavage activity of (Li+@C_{60})_n is much higher than that of (C_{60})_n, as shown in Fig. 6b, suggesting a cationic (Li+@C_{60})_n may electrostatically access to the minor groove in the double-stranded DNA.

In conclusion, highly dispersed (Li+@C_{60})_n produced by laser irradiation of Li+@C_{60} acts as an efficient photosensitiser for generation of singlet oxygen in an aqueous solution. The excited states of (Li+@C_{60})_n have been successfully detected by femto- and nanosecond transient absorption spectroscopies. We believe water-soluble (Li+@C_{60})_n can be employed as a convenient PDT photosensitizer in the near future.

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Notes and references

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Lithium ion encapsulated fullerene was solubilised to water by laser irradiation, acting as a photosensitiser for singlet oxygen generation.