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Fluorophore-capped cyclodextrins as efficient chemical-to-light energy converters

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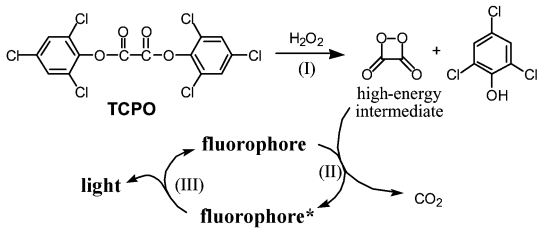
Bisbenzimidazole-capped cyclodextrins, capable of forming supramolecules, harvest chemical energy from the oxidation reaction of a bis(aryl)oxalate and emit light two orders of magnitude more efficiently than fluorescein does.

Cyclodextrins (CDs) are well-known to form inclusion complexes with a variety of guest molecules and to influence their photophysical and photochemical outcomes.[1] In connection with this, lots of fluorophore-appended CD derivatives and a few fluorophore-capped CD species have been reported. Inclusion of an energy acceptor in the CD cavity leads to a supramolecule[2] in which photo-induced energy transfer from the fluorophore appendage or cap to the bound guest has been extensively investigated.[3] However, using a cavity-bearing fluorophore to harvest energy generated in a chemical reaction is scarce.

A fluorophore can harvest energy from chemical or biotransformations. For example, a fluorophore can illuminate by hosting the chemical energy of oxidation intermediates of bis(aryl)oxalates, of which bis(2,4,6-trichlorophenyl) oxalate (TCPO) is the most prominent species. This approach is currently the most sensitive and versatile chemiluminescence detection method for liquid chromatography.[4] The overall reaction may be represented by Scheme 1: TCPO reacts with H_2O_2 to generate one or more highly energetic intermediates (step I) which excite a nearby fluorophore molecule (step II) and the latter fluoresces during relaxation (step III). Energy can be sidetracked along the way by losses in each step of the process, and the final outcome of light is dependent on the formation rate of the high-energy intermediates, excitation efficiency of the fluorophore and its emission quantum yield.

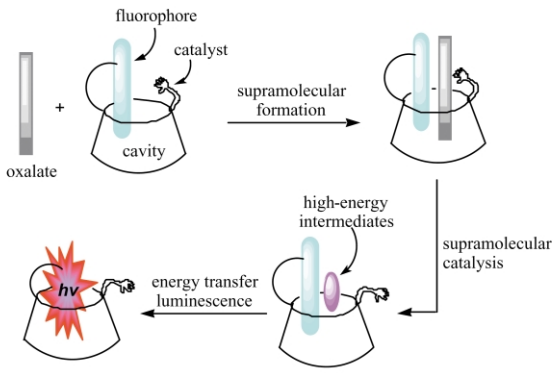
Imidazole is usually used to accelerate step I while the conventional fluorophores such as perylene, rubrene and fluorescein *etc*. are employed as energy acceptors to perform steps II and III. In these systems, the energy transfer (step II) is supposed to occur in the high-energy intermediate-fluorophore charge-transfer complex whose formation requires the collision of both species which is generally not very efficient. Most of the high energy intermediates undoubtedly decompose before they can undergo a bimolecular reaction with the fluorophore. It is reasonable to deduce that a fluorophore partaking of molecule recognition ability may bind the TCPO molecule and ensure the subsequent formation of the high-energy intermediates in the close vicinity of the fluorophore moiety within the supramolecule. Efficient supramolecular catalysis may be further expected by the proper immobilization of catalysts on the fluorophore-bearing host molecule (Scheme 2). We reason that such a supramolecular approach may contribute largely to the understanding of the overall luminescence process and also to the maximization of the light outcome by improving the efficiency of each step. The present paper describes the immobilization of fluorophores on CDs to effect high efficiency in harvesting chemical energy from the oxidation reaction of TCPO to fluoresce.



Scheme 1

The *p*-cresolbisbenzimidazole moiety is composed on the primary face of CDs by reacting 2-hydroxy-5-methyl-1,3-benzenedialdehyde with $6^A,6^C$-bis(*o*-aminoanilino)-α-CD and $6^A,6^D$-bis(*o*-aminoanilino)-γ-CD which are derived from α- and γ-CDs by disulfonylation at the primary face and subsequent displacement with *o*-phenylenediamine.[5] NMR spectral analysis† suggests that both **1** and **2** have a similar structure: the central cresol moiety of the aromatic cap is partially encased in the cavity with its methyl group directed inwards. Both **1** and **2** strongly emit around 490 nm in aqueous solutions when excited at 320 nm. Their ability to bind guest molecules is probed by using methyl orange as a quencher since its absorbance spectrum overlaps well the emission spectra of **1** and **2** and its absorbance around 320 nm is very low. The result of fluorescence titration experiments suggests that **2** and methyl orange form a very tight 1:1 inclusion complex with binding constant $K_a = 6.6 \times 10^4$ M^{-1}. Based on this result, strong binding of **1** and **2** with TCPO can be deduced though the binding constants were not directly determined because of the very poor water-solubility of TCPO.

Utilization of TCPO as an energy source to make **1** and **2** fluoresce affords very interesting results. Mixing fluorophore-TCPO (in 1:1 molar ratio) with H_2O_2 in a 10 mM imidazole solution buffered at pH 7.6 results in luminescence, and its intensity (relative chemiluminescence intensity, *i.e.* RCI is applied) increases as the concentration of fluorophore-TCPO is increased. A linear RCI–concentration relationship is observed for fluorescein. In sharp contrast to this, the RCI *vs.* concentration plots of CD fluorophore **1** demonstrate an upward curve at the early stage and gradually gain linearity at higher concentration range, indicative of the chemiluminescent importance of host–guest complexation[6] (Fig. 1a). The linear section means a complete complexation and its slope, an expression of relative chemiluminescene efficiency, is *ca.* 100 times that of fluor-



Scheme 2 Illustration of chemical-to-light energy conversion in a supramolecular manner.

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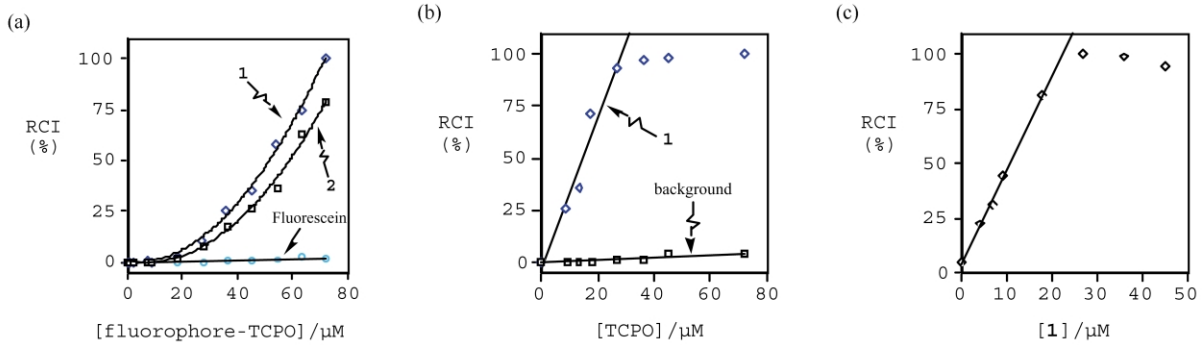
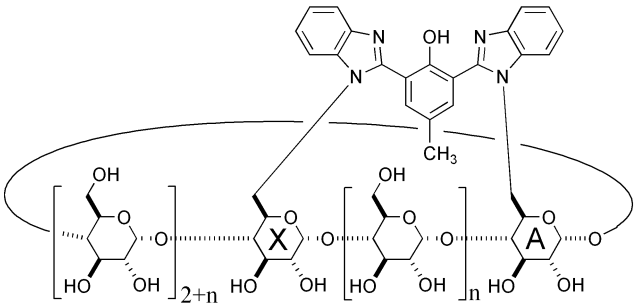


Fig. 1 Dependence of relative chemiluminescence intensity (RCI) on the concentration of fluorophores and/or TCPO. (a) 10 mM imidazole buffer at pH 7.6, 9 mM H_2O_2, [fluorophore] = [TCPO]. (b) 0.1 M phosphate buffer at pH 7.0, 9 mM H_2O_2, [**1**] = 27 μM. (c) 0.1 M phosphate buffer at pH 7.0, 9 mM H_2O_2, [TCPO] = 27 μM.



1: n = 1, X = C; **2**: n = 2, X = D

escein! Considering that the photoluminescence quantum yield of **1** is only 14% that of fluorescein, the excitation of **1** (concerning steps I and II in Scheme 1) is estimated to be *ca.* 700 times more efficient than that of fluorescein. This result undoubtedly indicates the importance of the conjugated CD moiety in the chemical energy transfer to the fluorophore even though the process from the initial fuel to the excited fluorophore cannot be drawn in detail at the present stage. Compound **2** behaves quite similarly to **1**, but with a slightly reduced RCI. Its chemiluminescence efficiency is deduced to be *ca*. 80% that of **1** by comparison of the slopes of their linear sections, which is consistent with the ratio of their fluorescence intensity.

Varying the molar ratio of the fluorophore/TCPO allows the elucidation of the stoichiometry of the complex. Since imidazole is known to bind with CDs and will compete against the fuel in binding, a phosphate buffer is used instead in order to increase the binding strength of the fluorophore and TCPO molecules. The result is somewhat astonishing. When the concentration of TCPO is varied from 0 to 72 μM with that of the fluorophore **1** fixed at 27 μM, the RCI increases and reaches a plateau at [TCPO] = 27 μM (Fig. 1b), ruling out any meaningful emission stemming from the oxidation of the fluorophore. By fixing the concentration of TCPO at 27 μM and increasing that of **1**, the RCI climbs rapidly at the first stage and ceases to further increase when the concentration of **1** exceeds 27 μM (Fig. 1c). These results strongly suggest the existence of a particularly tight 1:1 binding between **1** and TCPO. The binding complex is very important for luminescence (the bursting sections) while any excess of the individuals (free **1** and free TCPO) contribute insignificantly (the flat sections). That means the excitation of the fluorophore within the complex (*i.e.* intra-supramolecular energy transfer) is very efficient, whereas that between the complex and the high-energy intermediates generated from the free TCPO or between the free **1** and the high-energy intermediates generated from the complex (*i.e.* inter-supramolecular energy transfer) is very deficient. In addition, the fluorophore does not show obvious turnover (Fig. 1b, the flat section) probably because of the very tight binding and product inhibition.

The capped CDs **1** and **2**, though very efficient in converting chemical energy to light, by no means represent the best of the cavity-bearing fluorophores because of their lack of powerful catalytic functionalities to significantly accelerate the chemical transformations. In addition, their photoluminescence quantum yields are relatively low (10 ~ 14% that of fluorescein). Therefore, great potential exists to create cavity-bearing fluorophores with much higher efficiency in converting chemical energy to light.

In summary, this paper demonstrated for the first time that a fluorophore-capped CD is very efficient in harvesting excitation energy from a chemical reaction of its guest in a supramolecular way. Considering the enormous knowledge base surrounding CDs and other numerous host molecules for hydrophobic binding and enzyme mimicking, the novel concept presented herein may offer new opportunities in developing chemiluminescence systems of much higher efficiency.

Notes and references

† Spectral data of **2**: FAB-MS *m*/*z* 1601 [M + H], 1623 [M + Na]. ^{1}H-NMR (500 MHz, in DMSO-d_6, TMS int., deuterated): δ 7.76 ~ 7.25 (m, 10H, Ar-H), 5.30 (d, J = 3.9 Hz, 1H, H1), 5.09 (d. J = 4.1 Hz, 1H, H1), 4.91 (d, J = 3.7 Hz, 1H, H1), 4.82 (d. J = 3.7 Hz, 1H, H1), 4.80 (d, J = 3.7 Hz, 1H, H1), 4.75 ~ 4.44 (m, 7H, H1, H6A and H6D), 4.37 (m, 1H) and 4.12 (m, 1H) (H5A and H5D), 2.64 ~ 2.43 (m, overlapping with the signals of solvent, 7H, CH_3 and 2 pairs of H6), 3.75 ~ 2.92 (m, 38H, other protons). ^{13}C-NMR (125 MHz, in DMSO-d_6, TMS int.): δ 155.8, 150.4, 149.1, 140.6, 139.7, 137.0, 136.8, 131.1, 129.9, 126.0, 122.8, 122.6, 122.3, 122.2, 117.9, 117.8, 116.7, 115.5, 113.0 and 112.1 (Ar); 103.2, 102.6, 102.2, 102.1, 101.7, 101.6, 99.5 and 98.8; 85.4 and 85.2 (4A, 4D); 81.7, 81.5, 81.1, 81.0, 78.6 and 78.1 (4), 74.3, 73.5, 73.2, 73.0, 72.9, 72.8, 72.7, 72.4, 72.3, 72.1, 72.0, 71.9, 71.8, 71.7, 71.6, 71.5 and 71.3 (5, 3A ~ H, 2A ~ H); 71.9 and 68.5 (5A, 5D); 60.4, 59.9, 59.8, 59.4, 57.8 and 57.4 (6); 46.3 and 44.5 (6A, 6D), 20.6 (**C**H_3).

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