J Incl Phenom Macrocycl Chem (2007) 57:125–129
DOI 10.1007/s10847-006-9207-x

ORIGINAL ARTICLE

Coumarin-conjugated cyclodextrins: remarkable enhancement of the chemical-to-light energy transfer efficiency

De-Qi Yuan · Yuewei Zhao · Makoto Fukudome · Kahee Fujita

Received: 15 May 2006 / Accepted: 20 October 2006 / Published online: 18 January 2007

Abstract One or two coumarin units were incorporated to the primary face of γ-cyclodextrin (γ-CD), and the resultant coumarin derivatives were employed to harvest the chemical energy generated in the reaction of bis(trichlorophenyl)oxalate with hydrogen peroxide. In comparison with the coumarin without CD cavity for molecular recognition, the coumarin–CD conjugates demonstrated much higher chemiluminescence inetensity, indicating that the CD moiety remarkably improves the chemical energy transfer.

Keywords Cyclodextrin · Chemiluminescence · Hydrogen peroxide · Oxalate

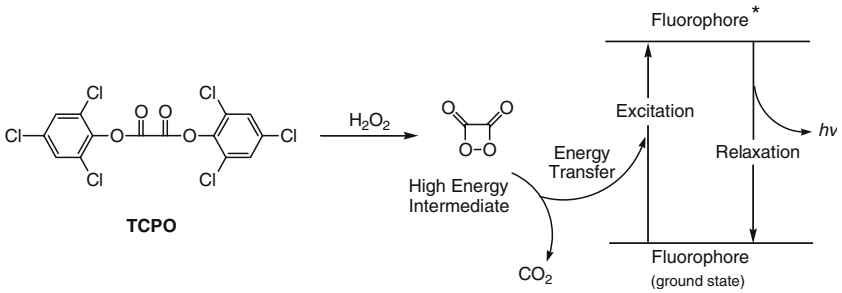
Introduction

A fluorophore can harvest energy from chemical or bio-transformations. 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 (CL) detection method for liquid chromatography [1] The overall reaction may be represented by Scheme 1: TCPO reacts with H_2O_2 to generate one or more highly energetic intermediates which excite a near-by fluorophore molecule and the latter fluoresces during relaxation. 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. Conventional fluorophores such as perylene, rubrene, fluorescein, etc. are employed as energy acceptors. In these systems, the energy transfer is supposed to occur by a bimolecular process which requires the collision of both species and is therefore generally not very efficient. Most of the high-energy intermediates undoubtedly decompose before they can undergo the bimolecular reaction with the fluorophore.

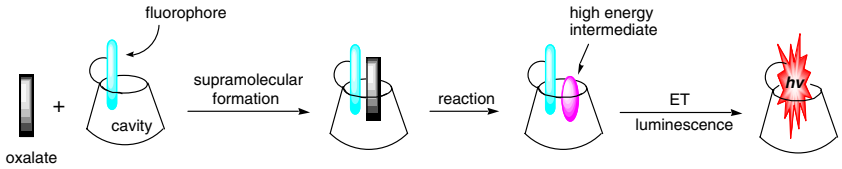
On the other hand, CDs are well known to form inclusion complexes with a variety of guest molecules and to influence their photophysical and photochemical outcomes [2]. In connection with this, lots of fluorophore-appended CD derivatives and a few fluorophore-capped CD species have been reported. Inclusion of an energy acceptor in the CD cavity leads to a supramolecule [3] in which photo-induced energy transfer from the fluorophore appendage or cap to the bound guest has been extensively investigated [4] However, using a cavity-bearing fluorophore to harvest energy generated in a chemical reaction is scarce [3]. It is reasonable to deduce that incorporation of a fluorophore to a molecule recognition site may ensure the formation of the high-energy intermediates in the close vicinity of the fluorophore moiety via the binding of TCPO prior to the chemical transformation and thus greatly improve the light outcome (Scheme 2). Recently, we demonstrated the first successful example of improving the total efficiency of the luminescence reaction of TCPO–H_2O_2 with a fluorophore-capped

D.-Q. Yuan (✉) · Y. Zhao · M. Fukudome · K. Fujita
Department of Molecular Medicinal Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Bunkyo-machi 1-14, Nagasaki 852-8521, Japan
e-mail: deqiyuan@nagasaki-u.ac.jp

Scheme 1



Scheme 2



CD [5]. The present paper describes the immobilization of coumarin on γ-CD to effect high efficiency of luminescence in the reaction of TCPO–H_2O_2.

Experimental

7-Hydroxycoumarin-4-acetic acid (coumarin), DCC, H_2O_2 and HOBT are from Sigma or Aldrich, and bis(2,4,6-trichlorophenyl)oxalate (TCPO) is from TCI. CD amines were prepared by the literature procedures. Condensation of the CD amines with 7-Hydroxycoumarin-4-acetic acid was performed in the presence DCC/HOBT. Phosphate buffer solution was prepared from KH_2PO_4 and NaOH with high-purity water. The pH value of buffer was accurately measured with a pH meter.

6-Deoxy-6-(7-hydroxycoumarin-4-acetamido)-γ-cyclodextrin (**3**)

6-Amino-6-deoxy-γ-CD (0.647 g, 0.5 mmol), 7-hydroxycoumarin-4-acetic acid (0.11 g, 0.5 mmol), DCC (0.412 g, 2 mmol) and HOBT (0.27 g, 2 mmol) were added into DMF (10 mL) and the resultant mixture was stirred at room temperature for 24 h. Then the reaction mixture was added to acetone (500 mL). The precipitates were collected by filtration and applied to chromatography on a reversed-phase Lobar column (RP-18, Size C). Elution of the column with a gradient from H_2O to 30% EtOH afforded the product **3** (0.58 g, 77%). TLC: R_f = 0.45 (on Merck precoated silica gel, developed with *n*-PrOH/AcOEt/H_2O = 7/7/5 (V/V/V)). Uv–vis: λ_{max} = 326 nm ($\varepsilon = 1 \times 10^4$); Fluorescence: λ_{em} = 460 nm; TOF-Mass: 1521 (M^+ + Na), 11537 (M^+ + K); ^{1}H-NMR (D_2O, 500 MHz, CH_3CN int.) (Fig. 1): δ = 2.99 (dd, $^3J(H_6,H_5)$ = 8.24 Hz, $^2J(H_6,H_6)$ = 14.42 Hz, 1H), 3.32~4.00 (m, 49 H), 4.96~5.06 (m, 8 H), 6.18 (s, 1 H), 6.52 (s, 1H), 6.71 (d, ^3J(H,H) = 8.69 Hz, 1H), 7.28 (d, ^3J(H,H) = 8.70 Hz, 1H); ^{13}C-NMR (D_2O, 500 MHz, CH_3CN int.) (Fig. 1): δ = 39.38 (CH_2 of coumarin), 41.57 (C_6), 60.95~61.06, 72.05~74.30, 79.86, 81.23~81.72 (m), 83.47, 101.19, 102.47~102.89, 104.03, 112.67, 113.14, 114.77, 126.61, 152.13, 152.21, 155.78, 162.01, 164.35 (C=O), 171.74 (NHC=O).

6^I,6^V-Dideoxy-6^I,6^V-bis(7-hydroxycoumarin-4-acetamido)- γ-cyclodextrin (**4**)

Similar procedure as described in the preparation of **3** was employed starting from 6^I,6^V-diamino-6^I,6^V-dideoxy-γ-CD **2** to give **4** as orange solids in 60% yield. TLC: R_f = 0.48 (on Merck precoated silica gel, developed with *n*-PrOH/AcOEt/H_2O = 7/7/5 (V/V/V)). Uv–Vis: λ_{max} = 324 nm ($\varepsilon = 1.9 \times 10^4$); Fluorescence: λ_{em} = 461 nm; TOF-Mass: 1721 (M^+ + Na), 1737 (M^+ + K); ^{13}C-NMR (DMSO-d_6, TMS int.) (Fig. 1): δ = 59.64~60.16 (m), 69.80, 71.79~73.08 (m), 80.22, 80.42, 81.09, 83.40, 101.25, 102.21, 111.34, 112,81, 126.60, 151.22, 154.83, 160.14 (C=O), 160.23 (C=O), 161.12 (NHC=O), 167.9 (NHC=O).

Chemiluminescence measurement

The whole CL reactions were carried out in buffered solutions (50 mM phosphate, pH = 7.0) and the detection of CL intensity was performed on a microlumatplus LB 96V auto-detector (Berthold technologies, Germany). The working solutions containing all

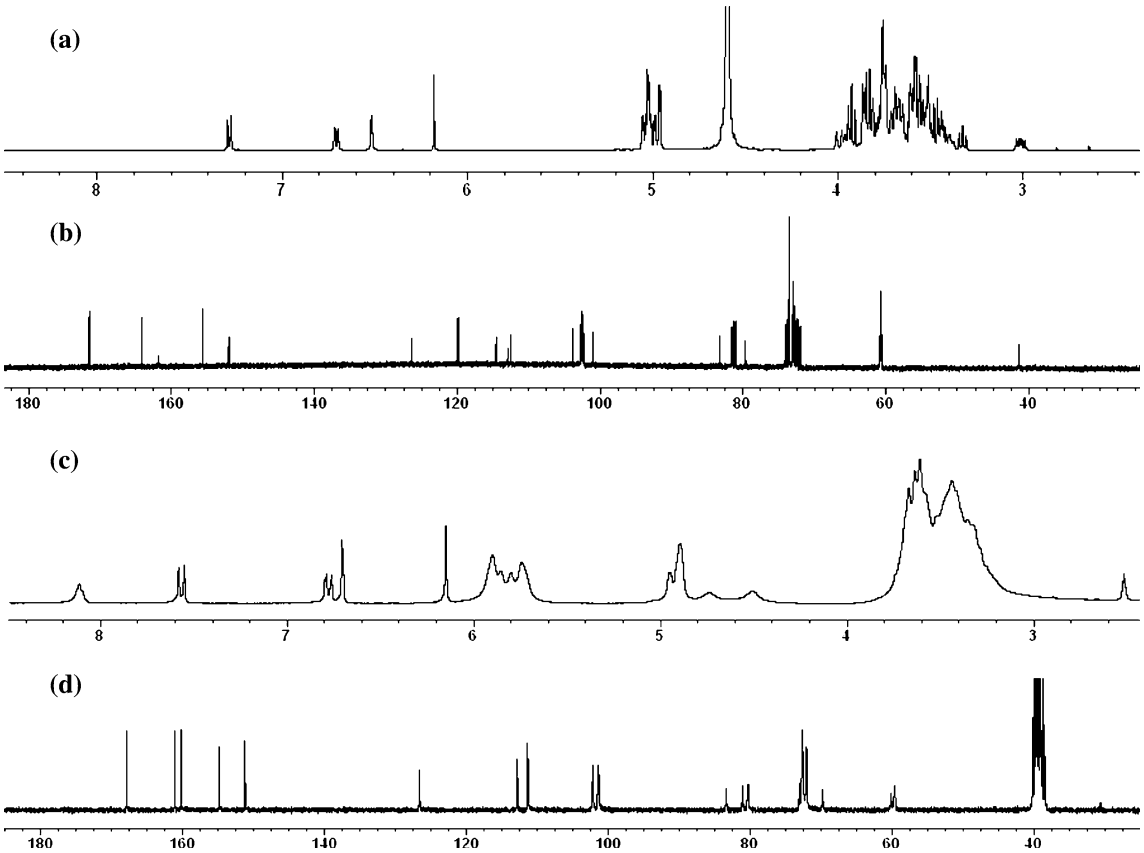


Fig. 1 ^{1}H and ^{13}C NMR spectra of coumarin-CDs **3** in D_2O (**a**, **b**) and **4** in DMSO-d_6 (**c**, **d**)

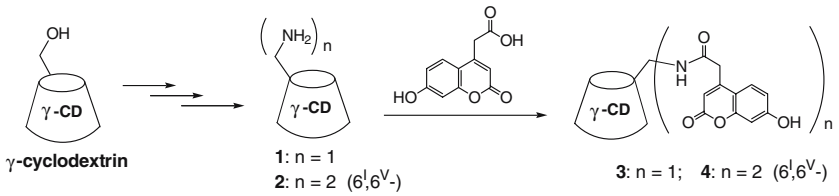
the necessary species except TCPO were added into the cells (300 μL) of a plate (12 × 8) in advance and sequential detection of CL was carried out. TCPO solution was auto-injected just before the detection. The apparent CL value originates from the integration of the light signal during the initial 120 s. All the CL values are the average of at least 4 parallel runs. For the control experiment, TCPO and H_2O_2 were used without fluorophores.

Result and discussion

The synthetic approach to the coumarin-CD conjugates is shown in Scheme 3. The CD amines **1** and **2** were prepared according to the literature procedures [6] by regioselective sulfonylation or disulfonylation of γ-CD, followed by subsequent displacement with NaN_3 and final reduction with Ph_3P. The condensation of the CD amines with 7-hydroxycoumarin-4-acetic acid were carried out in DMF in the presence of DCC and HOBT, affording the corresponding coumarin–CD conjugates **3** and **4** in moderated yields. The purity of the conjugates was confirmed by HPLC and there structures were characterized with NMR, MS, UV–vis and CD spectra.

The CL reaction was initiated by automatic injection of TCPO solution to the mixture of dye and H_2O_2 in a neutral phosphate buffer solution, and the collection of light over the whole spectrum was started immediately. As shown in Fig. 2, very strong light emission was detected at the beginning when compounds **3** and **4**

Scheme 3



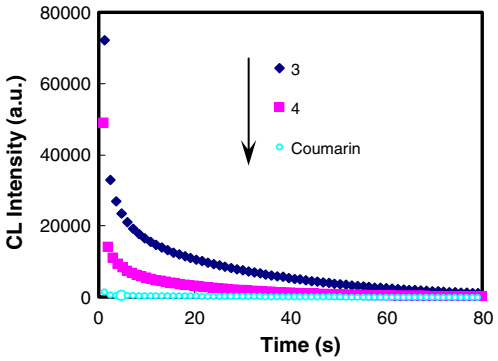


Fig. 2 The decay curves of chemiluminescence intensity [fluorophore] = 26.7×10^{-6} M, [TCPO] = 50×10^{-6} M, [H_2O_2] = 0.15 M

were used, and the light intensity decayed rapidly with reaction time. 7-Hydroxycoumarin-4-acetic acid, although gave some CL light, is much worse than the immobilized dyes **3** and **4**. The CL intensities in the absence of any one of TCPO and dye pigment were negligible compared with those of the coumarin–CD conjugates, ruling out any meaningful luminescence from the oxidation of the dye pigments. Upon varying the concentrations of the dyes and measuring the integrated emission intensity during the first 2 min, a linear relationship was observed between the CL intensity and dye concentration within the examined concentration range (Fig. 3). From Fig. 3, a ca. 20-fold increase in CL intensity was calculated for **3** relating to the un-immobilized coumarin. Interestingly, this value is half decreased by compound **4** although it has doubled dye units. These findings indicate that the immobilization of the dye on γ-CD greatly contributes to the total apparent CL amount.

According to the CL process depicted in Scheme 1, improvement of chemiexcitation of the fluorescence

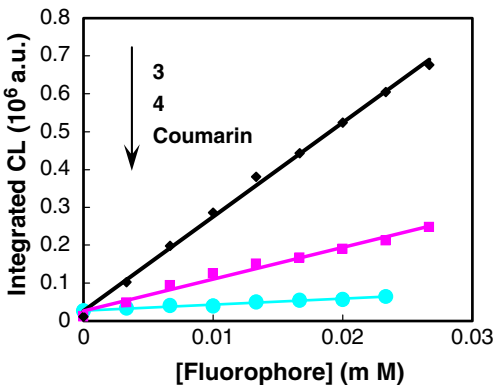


Fig. 3 Dependence of CL intensity on the concentration of fluorophores. [TCPO] = 50×10^{-6} M, [H_2O_2] = 0.15 M

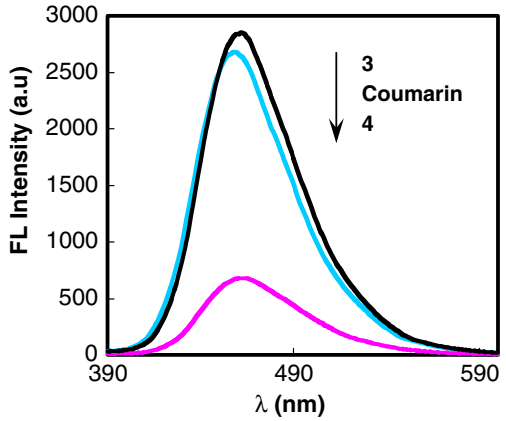


Fig. 4 Fluorescence spectra. λ_{ex} = 332 nm, [fluorophore] = 4×10^{-5} M

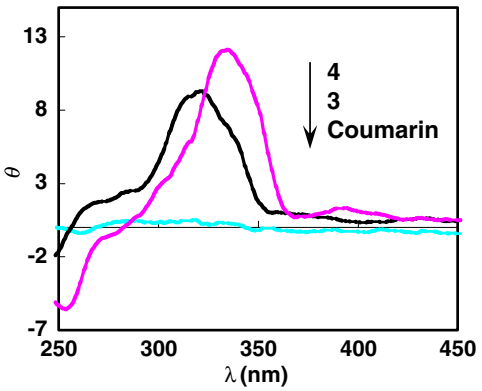


Fig. 5 The ICD spectra of the fluorophores. [fluorophore] = 4×10^{-5} M

dye to its excited states and/or the enhancement of the emission efficiency of the excited dye will lead to the increase of the total CL output. The relative emission efficiencies of the excited states of the various dyes were estimated by photo-fluorescence measurements. The fluorescence spectra (Fig. 4) show that immobilization of coumarin on γ-CD does not result in remarkable changes in photo quantum yield (dye **3**). It is therefore reasonable to deduce that the chemiexcitation of **3** is greatly improved and most likely the major origin of CL enhancement.

Compound **3** showed plus Cotton effect in the longer wavelength range around 340 nm in the induced CD spectrum (Fig. 5), implying that the coumarin moiety is not far apart from but most likely located (partially) in the cavity of CD. This conformation seems to be the desired structure for performing the energy transfer illustrated in Scheme 2.

In compound **4**, the second coumarin moiety failed to further increase the CL amount. Instead, it induced some decrease in CL intensity, which seems to result from the decrease of the luminescence efficiency by some intramolecular interaction between the two dye moieties (Figs. 4, 5).

Conclusions

Immobilization of coumarin moiety on γ-CD resulted in a ca. 20-fold increase in CL amount in the reaction of bis(trichlorophenyl)oxalate with hydrogen peroxide. The enhanced CL intensity is deduced to stem from the improvement of chemiexcitation of the dye. Simply increasing the number of dye moieties does not necessarily relate to CL enhancement because of the possible exciton coupling that causes significant quenching of luminescence.

References

1. Hadd, A.G., Lehmpuhl, D.W., Kuck, L.R., Birks, J.W., Mell, G.P.: Chemiluminescence demonstration illustrating principles of ester hydrolysis reactions. J. Chem. Educ. **76**, 1237–1240 (1999)
2. Bortolus, P., Marconi, G., Monti, S., Mayer, B., Kohler, G., Grabner, G.: Interaction of 4-hydroxybiphenyl with cyclodextrins: effect of complex structure on spectroscopic and photophysical properties. Chem. Eur. J., **6**, 1578–1591 (2000); Ramamurthy, V., Eaton, D. F.: Photochemistry and photophysics within cyclodextrin cavities. Acc. Chem. Res. **21**, 300–309 (1988)
3. Lehn, J.-M.: Supramolecular Chemistry. VCH, Weinheim (1995); Balzani, V., Scandola, F.: Comprehensive supramolecular chemistry. In: Atwood, J.L.J., Davies, E.D., MacNicol, D.D., Votle, F., Lehn, J.-M., Reinhoudt, D.N. (eds.) Photochemical and Photophysical Devices, vol. 10, pp. 687–746. Pergamon, Oxford (1996)
4. Tamura, M., Gao, D., Ueno, A.: A polyrotaxane series containing a-cyclodextrin and naphthalene-modified α-cyclodextrin as a light-harvesting antenna system. Chem. Eur. J. **7**, 1390–1397 (2001); Berberan-Santos, M.N., Choppinet, P., Fedorov, A., Jullien, L., Valeur, B.: Multichromophoric cyclodextrins. 8. Dynamics of homo- and heterotransfer of excitation energy in inclusion complexes with fluorescent dyes. J. Am. Chem. Soc. **122**, 11876–11886 (2000); Takakusa, H., Kikuchi, K., Urano, Y., Higuchi, T., Nagano, T.: Intramolecular fluorescence resonance energy transfer system with coumarin donor included in β-cyclodextrin. Anal. Chem. **73**, 939–942 (2001); Tabushi, I., Fujita, K., Yuan, L.C., Specific host-guest energy transfer by use of b-cyclodextrin rigidly capped with a benzophenone moiety. Tetrahedron Lett. 2503–2506 (1977)
5. Yuan, D.-Q., Kishikawa, N., Yang, C., Koga, K., Kuroda, K., Fujita, K.: Fluorophore-capped cyclodextrins as efficient chemical-to-light energy converters. Chem. Commun. 416–417 (2003)
6. Yuan, D.-Q., Tahara, T., Chen, W.-H., Okabe, Y., Yang, C., Yagi, Y., Nogami, Y., Fukudome, M., Fujita, K.: Functionalization of cyclodextrins via reactions of 2,3-anhydrocyclodextrins.J. Org. Chem. **68**, 9456–9466 (2003), and references therein