Cerium complexes of cyclodextrin dimers as efficient catalysts for luminol chemiluminescence reactions

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The chemiluminescence of a luminol–H_2O_2 system is found to be remarkably enhanced by the Ce^{IV} complexes of EDTA-bridged cyclodextrin dimers. The dimers were proved to work much more efficiently than the corresponding monomer. The cavity shape of cyclodextrin moieties and their cooperation displayed an important role in amplifying the chemiluminescence. Further modification of either the cyclodextrin rims or the EDTA linker altered significantly the catalytic abilities of the cyclodextrin dimers, and the examination of the effect of substituents on the chemiluminescence outputs suggested that the proximity between the cyclodextrin cavity and the metallic center might account for the amelioration of the chemiluminescence output.

Introduction

Cyclodextrins, a series of cyclic oligosaccharides consisting of 6 or more D-glucoside units linked together *via* α-(1–4)-glycoside bonds, are characteristic of a unique torus-shaped structure that comprises of an interior hydrophobic cavity to accommodate large varieties of chemical species ranging from organic molecules to inorganic ions.[1] The molecules included in the cyclodextrin cavity usually demonstrate chemical and physical properties quite different from those of their free forms in a bulky aqueous environment. Therefore, cyclodextrins are widely employed as molecular recognition sites to develop functional systems for diverse purposes,[2] among which the construction of cyclodextrin-based biomimetic materials[3] and molecular sensors[4] lies at the center of interest and has been witnessing great progress. Complex formation frequently results in changes in absorption/fluorescence properties,[4] but less frequently a change in chemiluminescence.[5] Cyclodextrins have also proved to be capable of mediating many organic transformations, either by providing a confined reaction field or by participating in the transformation of bound substrates with their functional groups.[6] Introduction of additional binding sites and/or functional groups into cyclodextrins improves greatly their catalytic ability or even results in the finding of novel functions.[7] Among the many cyclodextrin derivatives, cyclodextrin dimers and oligomers have attracted special interest.[8] Cooperation of the two or more cavities results in very strong host–guest binding while the functional groups on the linker that bridges cyclodextrin units together display excellent catalytic ability. Metal complexes on the linkers demonstrated large rate acceleration in the hydrolytic cleavage of carboxylate and phosphate esters,[9] and cyclodextrin tetramers with a metalloporphyrin core catalyzed oxidation of double bonds or saturated C–H bonds in a controlled manner[10] while selenium incorporated in the linker scavenged the active oxygen.[11]

On the other hand, luminol, when oxidized with hydrogen peroxide in aqueous alkaline solution, produces the excited state of phthalic acid, which emits light during relaxation (Scheme 1).[12] This reaction is widely used in the detection of hydrogen peroxide or the species that produce hydrogen peroxide. In general, the chemiluminescence is very weak, and enhancement of its intensity is of paramount importance.[13] Many metal ions and their complexes enhance the chemiluminescence of luminol. We envision that preorganization of luminol, hydrogen peroxide and the catalytic entity together may provide a possibility of improving the efficacy of the chemiluminescence (Scheme 2). Initially, we have chosen to examine cerium complexes of EDTA-bridged cyclodextrin dimers with the expectation to utilize the binding and catalytic properties of cyclodextrins to enhance the chemiluminescence of luminol.[14] Ceric ion was chosen because the ion itself does not alter the intensity of chemiluminescence,[15]

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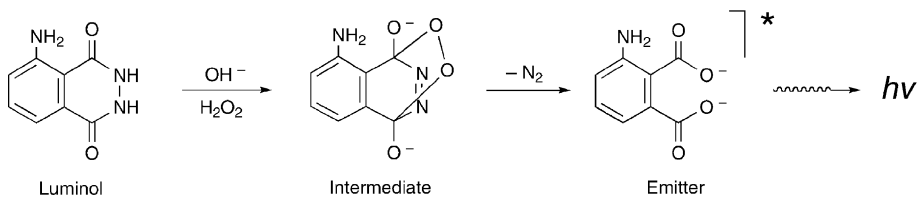
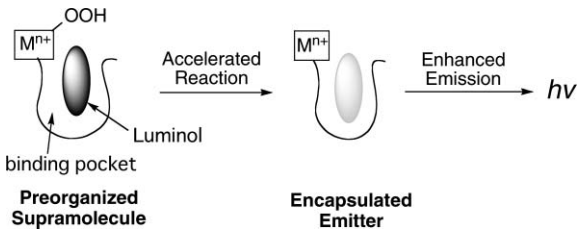


Scheme 1 The chemiluminescence reaction of luminol.



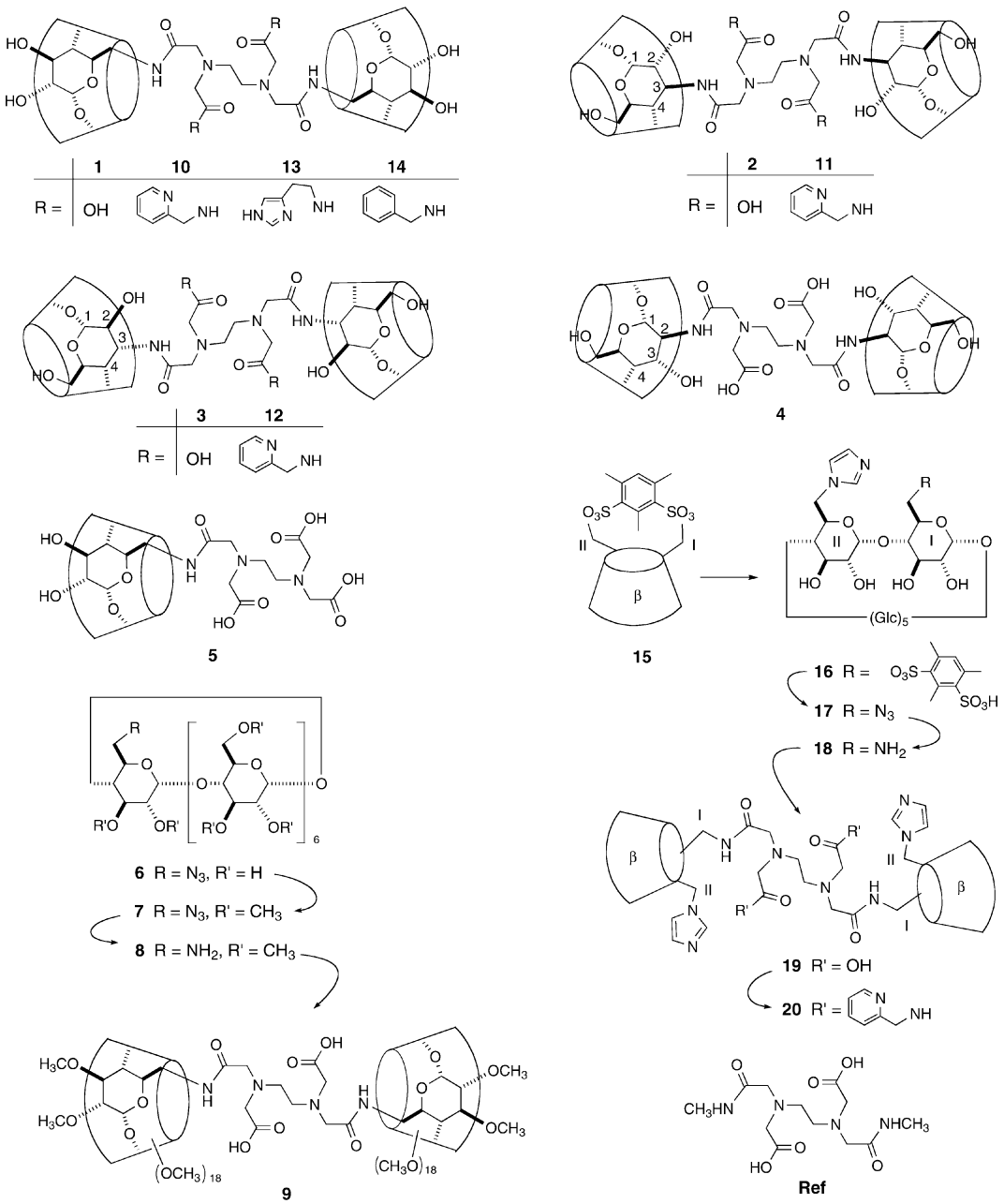
Scheme 2 Supramolecular formation to enhance the chemiluminescence efficacy.

which ensures a facile and reliable comparison of the experimental results.

Results and discussion

The syntheses and structures of the cyclodextrin derivatives employed in this research are depicted in Scheme 3.

EDTA-bridged cyclodextrin dimers **1–4** were prepared by the reported method from β-cyclodextrin.[16,17] EDTA-pendant β-cyclodextrin **5** and permethylated dimer **9** were obtained by the reactions of EDTA dianhydride with 6-amino-6-deoxy-β-cyclodextrin and permethylated 6-amino-β-cyclodextrin **8**, respectively. Reactions of dimers **1–3** with 2-picolylamine, histamine, and benzylamine in the presence of *N*,*N*′-dicyclohexyl carbodiimide (DCC) and *N*-hydroxylbenzotriazole (HOBt) at rt for 3 days afforded dimers **10–14** in 40–75% yields, respectively. An imidazolyl group was also attached directly to the rim of



Scheme 3 Syntheses and structures of cyclodextrin derivatives utilized in the chemiluminescence reaction of luminol.

the cyclodextrin moiety (Scheme 3). Two adjacent 6-hydroxyl groups of β-cyclodextrin were first activated by reacting with 1,3-mesitylenedisulfonyl in pyridine and the resultant disulfonate (**15**)[18] was then opened with imidazole to generate the heterobifunctional β-cyclodextrin **16**. This method also produced a minor amount of the counterclockwise isomer (4.8% based on isolated disulfonate **15**). The major isomer **16**, whose regiochemistry was elucidated by enzymatic degradation of the cyclodextrin ring together with EI-MS analysis of the degradation product,[18] was converted to 6^{I}-amino-6^{II}-imidazolyl-β-cyclodextrin **18** by substitution of the remaining sulfonate group with sodium azide and subsequent reduction with triphenylphosphine. Reaction of **18** with EDTA dianhydride generated dimer **19** which was further converted to **20** with 2-picolylamine. Compounds **19** and **20** represent the first examples of cyclodextrin dimers with functional groups other than OH being attached on the cyclodextrin rims instead of being incorporated to the linker. All the new compounds were characterized with MS and NMR spectra.

Cerium complexes[19] of the cyclodextrin dimers were obtained by mixing the aqueous solution of cyclodextrin dimers with a freshly prepared aqueous solution of $Ce(NH_4)_2(NO_3)_4$, and their catalytic properties were tested on the chemiluminescence reactions of luminol. To 100 μl of 0.1 M Na_2CO_3 solution (pH 11.5) were successively added 10 μl of 1.0×10^{-5} M luminol solution, 40 μl of 0.25 M H_2O_2, and then 50 μl of 0.5 mM Ce^{IV}-complex solution. As soon as the addition was finished, the resultant solution was mixed on an auto-mixer, and the measurement of light output was immediately started. For a typical run, the time interval was about 5 s between the mixing of components and chemiluminescence measurement. Light outputs over the entire spectrum were collected and the integrated intensity of the initial minute was employed for the characterization of the chemiluminescence efficiency of each catalyst. All the data of the chemiluminescence intensity were the averages of three independent measurements. Neither obvious change in pH nor precipitation of Ce^{IV} species were observed during the chemiluminescence measurements, except in the case of Ce^{IV} without ligands.

Fig. 1 shows the chemiluminescence decays of luminol in the presence and absence of catalysts. Luminol was almost chemiluminescently unreactive in the absence of catalysts. None of cyclodextrin dimer **1**, Ce^{IV} ion and EDTA-Ce^{IV} complex demonstrated obvious influence on the chemiluminescence reaction. However, as soon as the **1**-Ce^{IV} complex was used, the emission of luminol was remarkably enhanced. This increase in light output is not associated with any changes in pH, since no meaningful difference in pH was observed between the various reaction mixtures, and the pH values remained constant during the chemiluminescence measurements. The chemiluminescence spectrum (Fig. 2) of this reaction, which was recorded on a fluorescence spectrometer without the use of an excitation source, displayed an emission band centered at 460 nm, a reasonable region for luminol chemiluminescence. Both the excitation and fluorescence spectra of the total reaction products were consistent with those of the 3-aminophthalate anion at the same pH. These facts confirmed that the chemiluminescence originated from the reaction of luminol. Interestingly, the chemiluminescence mixima was about 35 nm red-shifted compared to that of the photo-induced fluorescence, implying chemiluminescence has a more stabilized excited state (by *ca.* 0.2 eV). The stabilization is reasonably considered to relate to the special microenvironment where the emitters were generated: in close proximity to the metal center and probably in interactions with cyclodextrin moieties (*vide infra*). These results are indicative of the importance of the **1**-Ce^{IV} complex as a catalyst. The complex formation is found to take a few minutes to complete and this enabled the examination of activity–time dependence of the mixture of **1** and Ce^{IV} freshly prepared from their individual stock solutions. The test showed the catalytic ability of the mixture increased rapidly, together with the mixing time, and approached a constant value in about 5 minutes after the mixing of the individual solutions of Ce^{IV} and **1**, which is further evidence of the importance of the complex as a catalyst.

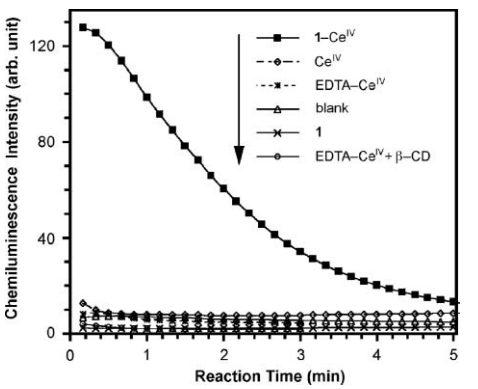


Fig. 1 Chemiluminescence decays in the luminol chemiluminescence reactions. Reaction conditions: 100 μl of 0.1 M aqueous Na_2CO_3 (pH 11.5), 10 μl of 1.0×10^{-5} M luminol, 40 μl of 0.25 M H_2O_2, and 50 μl of 5.0×10^{-4} M catalyst solutions were mixed and light collection was started immediately.

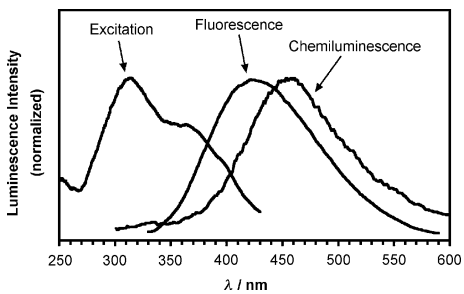


Fig. 2 Chemiluminescence spectrum of luminol in the presence of the cerium complex of **1** and the fluorescence (λ_{ex} = 305 nm) and excitation spectra of the total reaction products.

Many metal ions are known to catalyze this reaction, increasing the light emission or at least speeding up the formation of the emitter and therefore the onset of light production.[13] It was also reported that the binding of luminol to hydrophobic regions could strongly enhance the chemiluminescence.[20] However, the present system is different from those cases in that it combines both a hydrophobic binding site and the catalytic metal complex center in a single molecule just as most metalloenzymes do. It is therefore the covalent linkage and cooperation of β-cyclodextrin and EDTA-Ce^{IV} components that are essential for the catalytic ability since neither the component individuals nor their mechanical combination resulted in obvious changes in chemiluminescence.

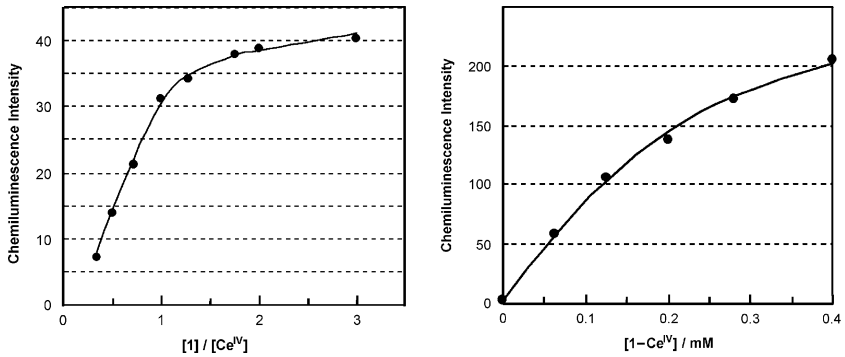


Fig. 3 The dependence of chemiluminescence intensity on the [**1**]–[Ce^{IV}] ratio (left, final [Ce^{IV}] = 6.25×10^{-5} M, [**1**] = 2.08×10^{-5}–1.88×10^{-4} M) and [**1**-Ce^{IV}] (right, final [**1**-Ce^{IV}] = 0–4.00×10^{-4} M). Reaction conditions: 100 μl of 0.1 M aqueous Na_2CO_3 (pH 11.5), 10 μl of 1.0×10^{-5} M luminol, 40 μl of 0.25 M H_2O_2, and 50 μl of catalyst solutions were mixed and light collection was started immediately.

Alteration of the molar ratio of [**1**]–[Ce^{IV}] allows the elucidation of the components of the catalytically active species. When the concentration of dimer **1** is varied from 20.8 to 188 μM with that of the Ce^{IV} fixed at 62.5 μM, the chemiluminescence intensity increases rapidly at the first stage and reaches a plateau when the ratio of [**1**]–[Ce^{IV}] exceeds 1 (Fig. 3, left). These results strongly suggest that the 1 : 1 complex **1**-Ce^{IV} is the active species for catalyzing the chemiluminescence reaction.

We also examined the influence of catalyst concentration on the chemiluminescence intensity. Upon varying [**1**-Ce^{IV}] from 0 to 0.4 mM, the chemiluminescence intensity increased proportionally at a lower concentration range. Although a complete saturation curve was not obtained because of the limited solubility of the Ce^{IV} complex, obvious downward deviations were observed at a higher catalyst concentration range (Fig. 3, right), which is indicative of the existence of the expected substrate–catalyst binding.

The cavity shape and its geometry related to the linker (Ce^{IV} complex center) remarkably influence the catalytic ability. When dimer **2** was used instead of **1**, chemiluminescence intensity decreased to one fifteenth of that detected in the case of dimer **1** (Fig. 4). This result is somewhat astonishing because dimers **1** and **2** differ only in the positions at which the EDTA linker is attached to the cyclodextrin moieties. Dimer **2** has cyclodextrin cavities of the same shape as, but in an opposite arrangement to those of dimer **1**. Chemiluminescence measurements indicated that, this difference in cavity arrangement in **2** resulted in a dramatic loss of catalytic ability. Dimer **3**, which has the same cavity arrangement as dimer **2** (linker at C-3) but a slight distortion in cavity shape due to the inversed configurations of C-2 and C-3 carbons,[21] demonstrated 4 times the enhancement in catalytic ability compared to that of dimer **2**. Dimer **4**, though quite similar to dimer **3** in cavity structure and cavity arrangement, is 3 times less efficient than dimer **3**. Quite interestingly, the monomer **5** is only one tenth as efficient as the corresponding dimer **1**. These results clearly indicated that the dimer structure is preferred for the chemiluminescence reaction and any small structural changes in the cyclodextrin moieties might cause obvious influences on the catalytic ability of cyclodextrin dimers.

Since cyclodextrins are known to bind a variety of organic molecules, it is reasonable to deduce that the cyclodextrin moieties interact with luminol.[1] ^{1}H NMR spectroscopy was used to investigate the substrate–catalyst interaction. Addition of luminol to the D_2O solution of dimer **1** did not cause obvious changes of the ^{1}H signals of **1**, indicating that dimer **1** may not bind luminol strongly. However, the mixture of luminol and **1**-Ce^{IV} complex demonstrated a much more complicated ^{1}H NMR spectrum than that of **1** (Fig. 5). The signals relating to the EDTA linker were significantly shifted, most likely by the coordination of Ce^{IV}. More importantly, the signals relating to the sugar part became more extensively resolved, implying that the differences between the sugar units were magnified upon binding luminol and Ce^{IV}. Although the complicity of the spectrum and of the poor solubility of luminol prevented meaningful spectral assignments and further structural elucidation, the significant spectral changes should indicate that luminol coordinates to the Ce^{IV} and binds

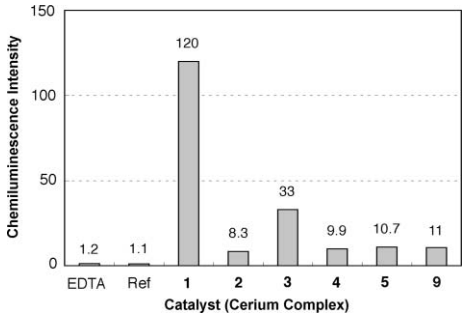


Fig. 4 Influence of cyclodextrin structure on the chemiluminescence intensity. Reaction conditions: the same as described in Fig. 1.

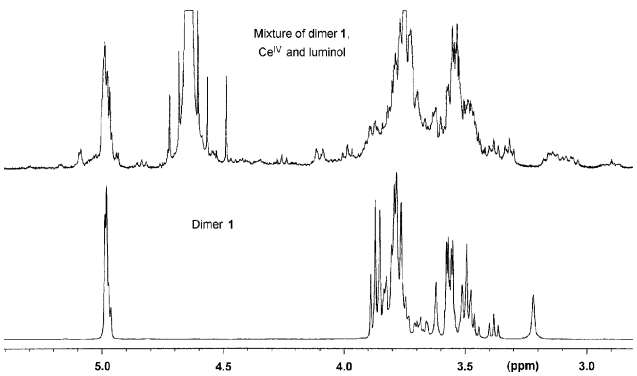
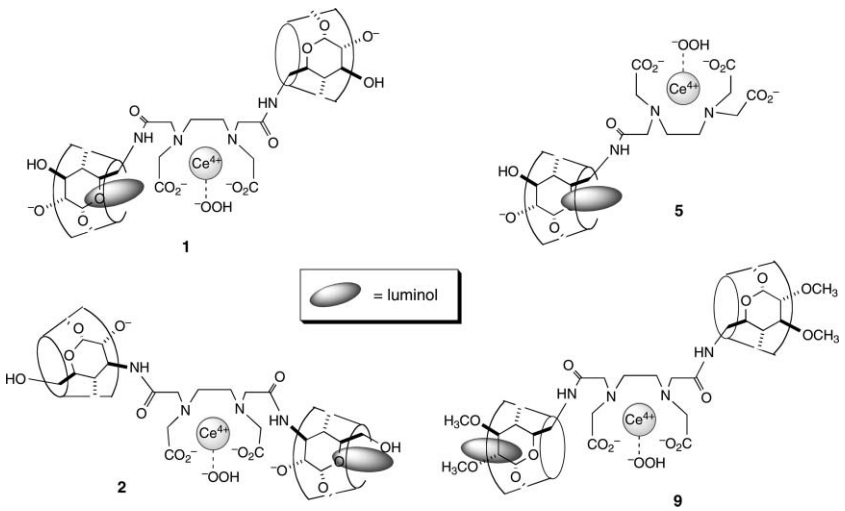


Fig. 5 ^{1}H NMR spectra of dimer **1** in the presence (top) and absence (bottom) of luminol and Ce^{IV} in D_2O.



Scheme 4 Plausible pre-organization of luminol and oxidant by cyclodextrin dimers.

simultaneously the cyclodextrin either partially entering the cavity or just spanning over its entrance.

Based on the above results, we tentatively propose a pre-organization of the two reactants by the catalyst (Scheme 4) for the catalyzed chemiluminescence reaction: the Ce^{IV} center binds the HOO^- while the cylodextrin hydrophobic cavity, together with the Ce^{IV}, brings luminol close to the bound HOO^-, thus the local concentrations of both luminol and oxidant are greatly increased. The chemiluminescence is switched on only when the metal center and the hydrophobic cavities can efficiently cooperate, which means the geometry of cyclodextrin dimers would be very important and it was proved to be the case by dimers **1** and **2**. The remarkable difference in catalytic ability of these two dimers can be rationalized as follows. The secondary side of cyclodextrin is less hydrophobic but more acidic. Under the experimental conditions, it will partially ionize and become even worse at accommodating luminol. On the other hand, the primary side is more hydrophobic and will remain un-ionized at the experimental pH and, therefore, is preferential for binding luminol. Based on this consideration, dimer **1** is expected to locate luminol towards the center of the molecule where the oxidant is bound, whereas **2** directs luminol outwards to either terminal of the molecule. In addition, the ionized secondary OH groups of **2** may compete with HOO^- in coordinating to the Ce^{IV} on the same side. Actually, all the cyclodextrin dimers (**2–4**) bridged at the secondary hydroxyl side are much less efficient than dimer **1**. Breslow *et al.* demonstrated that in heptakis(6-methylamino)-β-cyclodextrin, the methyl groups were inserted inwards to the cyclodextrin cavity, forming a floor.[22] It is reasonable to deduce that permethylation of dimer **1** would inverse the orientation of luminol, that is, the luminol molecule would be accommodated at the secondary side instead of the primary side near the Ce^{IV} center. Indeed, the permethylated dimer **9**-Ce^{IV} only showed one tenth of the catalytic activity of **1**-Ce^{IV}. The 10-fold higher efficacy of dimer **1** compared to that of corresponding monomer **5** implies that the second cyclodextrin should play a more important role than just increasing the probability of substrate-binding and exercising the simple effect of substitution. It is likely that the second cyclodextrin may greatly reduce the geometrical mismatch between the oxidant and cyclodextrin cavity where the substrate is bound. The pseudo *cis*- and *trans*-conformers with the oxidant, being situated close to or far away from the cyclodextrin cavity, respectively, represent the two typical conformations among the many possible conformations of the Ce^{IV} complex of **5**. It is reasonable to deduce that the catalytically non-productive *trans*-conformer is thermodynamically favored over the catalytically productive *cis*-conformer. However, the situation of dimer **1** is quite different. In the thermodynamically favored conformation, the two cyclodextrin moieties would be "*anti*-" to each other and one of them would be *cis*- (close) to the bond oxidant. That is, the catalytically productive conformer is thermodynamically favored.

Apart from the catalytic effect, the cyclodextrin dimers might also alter the chemiluminescence intensity by protecting the excited molecules from self-annihilation and dynamic quenching. We measured the effect of the **1**-Ce^{IV} complex on the fluorescence intensity of 3-aminophthalic acid, which is believed to be the emitter in the luminal chemiluminescence reaction. Under similar conditions to that of the chemilunescence reaction (but without H_2O_2), the increase of the concentration of the **1**-Ce^{IV} complex caused a decrease of the fluorescence intensity. This observation implies that the **1**-Ce^{IV} complex slightly quenches the fluorescence instead of protecting the excited 3-aminophthalate from non-radiative relaxation. Although there might be some differences between the excited states produced chemically and photochemically,[23] the large enhancement of the chemiluminescence intensity, together with the fluorescence quenching results, undoubtedly indicated that the principal effect of the **1**-Ce^{IV} complex should be the mediation of the chemical transformations to generate the excited products.

Modification of the carboxylic acid groups and cyclodextrin rims of the dimers gave interesting results. As shown in Fig. 6, introduction of pyridyl groups to the linker of dimers **1–3** obviously improved the chemiluminescence (dimers **10–12**), with the largest increase of 5.4-fold in chemiluminescence intensity being observed for dimer **11**. In contrast, imidazolyl groups caused dramatic loss of catalytic ability of the dimers. Dimer **13**, which has two imidazolyl groups on the linker, demonstrated only one-fourth of the catalytic activity of the corresponding diacid **1**. Even lower

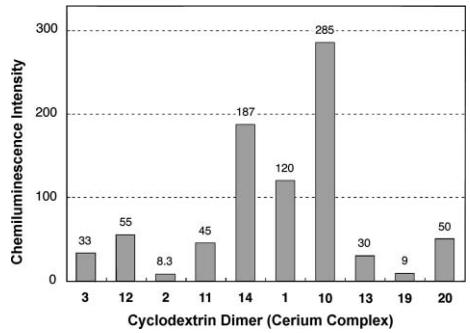


Fig. 6 Influence of substituents on the chemiluminescence intensity. Reaction conditions: the same as described in Fig. 1.

activity was observed when imidazolyl groups were introduced directly to the cyclodextrin rims (dimer **19**). Modification of the carboxylic groups of dimer **19** with picolylamine greatly improved the catalytic ability (dimer **20**), but dimer **20** was still 5 times less efficient than the corresponding imidazolyl-free dimer **10**. Although a ligand may alter the coordination environment and therefore affect the catalytic property of the metal complex, such a large difference between imidazolyl and pyridyl groups is still somewhat surprising. It seems that hydrophobicity of the substituents plays an important role, since replacement of pyridyl groups with the non-coordinative phenyl groups (dimer **14**) still gave a high chemiluminescence intensity.

Comparison of the structure and activity of dimer **11** with those of dimer **12** afforded some insight on the function of the pyridyl substituents. Fig. 7 shows the chemical shifts of the pyridyl protons of dimers **11** and **12**. Even though the pyridyl groups are separated by as many as 9 single bonds from cyclodextrin skeletons where the only structural difference between dimers **11** and **12** appears, their chemical shifts are quite different from each other. Dimer **11** not only demonstrated much lower chemical shifts for the H-6, H-5 and H-4 protons (δ_{11}–$\delta_{12} = -0.03$, -0.1 and -0.07, respectively), but inversed the order of H-3 and H-5 as well. This observation implies that the pyridyl groups of dimer **11** are subjected to stronger alicyclic shielding effects from cyclodextrin cavities[24] and, therefore tighter self-inclusion complexation could be deduced. ROESY experiments demonstrated strong NOE cross-correlations between all the protons of 2-picolyl groups (including the methylene protons) and the inner H-3 and H-5 protons of cyclodextrin moieties (Fig. 8). This stronger self-inclusion may contribute to the 5.4-fold increase of chemiluminescence intensity, which is rather large when compared with the 0.7-fold increase of

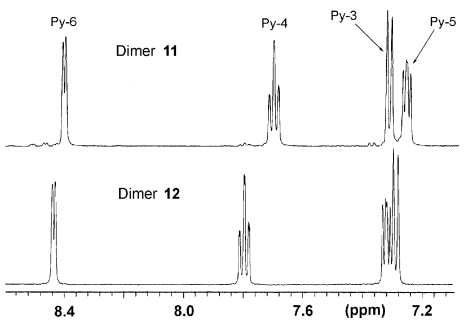


Fig. 7 Partial ^{1}H NMR spectra of dimers **11** and **12** in D_2O.

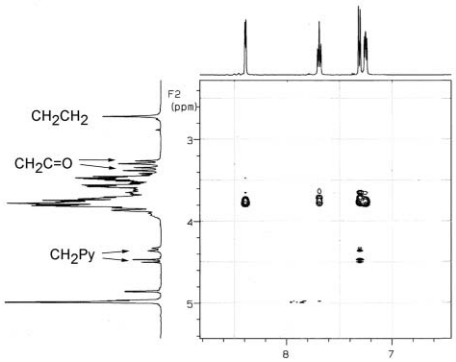


Fig. 8 Partial ROESY spectrum of dimer **12** in D_2O.

dimer **12**, probably by shortening the distance between the Ce^{IV} center and the cyclodextrin cavities.

Conclusion

In summary, we have described the synthesis of a series of ETDA-bridged cyclodextrin dimers and demonstrated that the cerium complexes of these dimers could enhance the chemiluminescence intensity of luminol by pre-organizing the hydrogen peroxide and luminol together. The appropriate cavity arrangement and cavity shape proved to be very important for improving the chemiluminescence. The dimer bridged at the primary hydroxyl side was one order of magnitude more efficient than those bridged at the secondary hydroxyl side. Changes in the cavity shape of the dimers also significantly influence the chemiluminescence intensity. Modification of either the cyclodextrin rims or the EDTA linker altered significantly the catalytic abilities of the cyclodextrin dimers.

Experimental

Reversed-phase column chromatography was performed on Merck prepacked Lobar columns (LiChroprep® RP-18, size B or size C). Thin layer chromatography (TLC) was carried out on Merck aluminium-backed 0.2 mm silica gel 60F-254 plates with mixed solvents of *n*-propanol–ethyl acetate–water in volume ratios of 7 : 3 : 6 (solvent A), 7 : 3 : 7 (solvent B) as the mobile phase.

Permethylated β-cyclodextrin dimer 9. A mixture of 6-azido-6-deoxy-β-cyclodextrin **6** (300 mg, 0.26 mmol), sodium hydride (750 mg, 31 mmol) in dry DMF (10 ml) was sonicated for 15 min in a water bath. Methyl iodide (3 ml) was then added and sonication was continued for an additional 15 min. The reaction mixture was diluted with water and extracted with dichloromethane. After the organic solvent was evaporated, the residue was subjected to column chromatography on silica gel. Elution of the column with CH_2Cl_2 (75 ml), then CH_2Cl_2–EtOH (20 : 1; 180 ml) and finally CH_2Cl_2–EtOH (10 : 1; 500 ml) afforded permethylated 6-azido-6-deoxy-β-cyclodextrin **7** (224 mg, 60%). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 98.0, 97.9, 97.7, 97.6, 97.5, 81.5, 81.3, 81.0, 79.6, 79.5, 79.3, 71.0, 70.3, 70.2, 60.8, 60.6, 60.5, 58.1, 58.0, 57.9, 57.8, 57.7, 57.5, 51.3; FAB-MS: *m/z* 1440 (M + H). The azide **7** (100 mg, 0.069 mmol) and triphenylphosphine (90 mg, 0.34 mmol) were dissolved in dry DMF (5 ml) and the mixture was stirred at rt. Two hours later, aqueous ammonia (28%, 0.8 ml) was added

and the reaction mixture was stirred at rt for 1 d. After dilution with 5% ethanol solution and membrane filtration, the aqueous solution was subjected to chromatography on a reversed-phase Lobar column (size B) with gradient elution from 60% aqueous ethanol (500 ml) to ethanol (500 ml) to afford the corresponding permethylated β-CD amine **8** (51 mg, 52%; FAB-MS, *m/z* 1414 (M + H)). A solution of β-CD amine **8** (48 mg, 0.034 mmol) and EDTA dianhydride (3 mg, 0.012 mmol) in DMF (1 ml) was stirred at rt for 2 d. Water (5 ml) was then added and the reaction mixture was heated at 80 °C for 5 h. After evaporation of the solvents under reduced pressure, the residue was subjected to column chromatography on silica gel. Elution of the column with CH_2Cl_2–CH_3OH (3 : 1) afforded permethylated β-cyclodextrin dimer **9** (17 mg, 32%). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 173.1, 171.3, 97.9, 97.8, 97.6, 81.5, 81.4, 81.1, 79.8, 79.5, 79.4, 71.0, 70.3, 70.2, 69.7, 60.6, 58.3, 58.1, 57.6, 57.5, 52.1. ^{1}H-NMR (DMSO-d_6, TMS int.): δ 5.19 (d, ^3J = 3.8 Hz, 2H), 5.10 (d, ^3J = 3.8 Hz, 2H), 5.06 (m, 10H), 3.79–3.65 (m, 28H), 3.60–3.20 (m, 85H), 3.15–2.95 (m, 18H). FAB-MS: *m/z* 3083 (M + H).

General procedure for the modification of the EDTA linkers of β-cyclodextrin dimers 10–14. β-Cyclodextrin EDTA dimer (600 mg, 0.24 mmol), DCC (148 mg, 0.72 mmol), HOBt (96.3 mg, 0.71 mmol) and an amine (1.90 mmol) dissolved in dry DMF (5 ml) were stirred at rt for 60 h. Water (5 ml) was then added and the resultant mixture was heated at 80 °C for an additional 5 h. After dilution with water (400 ml), the mixture was filtered and the filtrate was concentrated to a volume of *ca.* 5 ml. The residues were slowly added to acetone (500 ml) under stirring to precipitate cyclodextrin species. Dimers **10–14** were purified by chromatography of the precipitates on a reversed-phase Lobar column (size B, gradient elution from water to 45% methanol) affording the modified cyclodextrin dimers.

Dimer 10. Prepared from the reaction of **1** with 2-picolylamine. Yield: 38%. R_f = 0.14 (solvent B). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 170.8, 170.7, 158.2, 148.6, 136.6, 121.9, 120.9, 102.1, 101.9, 101.7, 83.2, 81.8, 81.6, 81.5, 81.4, 81.3, 73.0, 72.9, 72.7, 72.3, 72.1, 71.9, 69.4, 60.0, 59.9, 59.8, 58.0, 57.9, 52.7, 43.9, 40.1. ^{1}H-NMR (DMSO-d_6, TMS int.): δ 8.60 (t, ^3J = 5.9 Hz, 2H), 8.47 (d, ^3J = 4.5 Hz, 2H), 7.80 (br. s, 2H), 7.72 (dt, ^3J = 7.7, 1.6 Hz, 2H), 7.25 (d, ^3J = 8.0 Hz, 2H), 7.23 (dd, ^3J = 7.7, 2.3 Hz, 2H), 5.74–5.60 (m, 28H), 4.86–4.81 (m, 14H), 4.44–4.35 (m, 16H), 3.80–3.50 (m, 56H), 3.37–3.15 (m, 36H), 2.70 (br. s, 4H). FAB-MS: *m/z* 2705 (M + H).

Dimer 11. Prepared from the reaction of **2** with 2-picolylamine. Yield: 70.5%. R_f = 0.14 (solvent B). ^{13}C-NMR (D_2O, CH_3CN int.): δ 175.2, 175.1, 157.9, 149.1, 138.8, 123.7, 122.9, 102.9, 102.8, 102.7, 102.6, 102.4, 102.3, 82.6, 82.1, 82.0, 81.9, 81.8, 78.8, 74.2, 74.1, 74.0, 73.8, 73.0, 72.8, 72.7, 72.5, 72.3, 70.8, 61.2, 61.1, 59.6, 59.1, 55.1, 54.2, 44.8. ^{1}H-NMR (D_2O, CH_3CN int.): δ 8.40 (d, ^3J = 4.7 Hz, 2H), 7.70 (t, ^3J = 7.7 Hz, 2H), 7.31 (d, ^3J = 7.9 Hz, 2H), 7.25 (dd, ^3J = 6.1, 1.7 Hz, 2H), 4.99 (m, 12H), 4.86 (d, ^3J = 3.5 Hz, 2H), 4.48 (d, ^3J = 15.9 Hz, 2H), 4.34 (d, ^3J = 15.9 Hz, 2H), 3.93–3.41 (m, 84H), 3.41–3.26 (m, 8H), 2.72 (br. s, 4H). FAB-MS: *m/z* 2705 (M + H).

Dimer 12. Prepared from the reaction of **3** with 2-picolylamine. Yield: 57%. R_f = 0.14 (solvent B). ^{13}C-NMR (D_2O, CH_3CN int.): δ 174.7, 174.2, 157.9, 149.4, 139.1, 123.8, 122.6, 104.1, 102.7, 102.6, 102.6, 102.4, 102.2, 81.9, 81.8, 81.7, 80.5, 74.3, 74.2, 74.1, 74.0, 73.9, 73.2, 72.9, 72.8, 72.7, 72.6, 72.4, 72.2, 61.2, 61.1, 60.5, 59.3, 59.2, 54.1, 51.5, 45.2. ^{1}H-NMR (D_2O, CH_3CN int.): δ 8.43 (d, ^3J = 4.7 Hz, 2H), 7.80 (dt, ^3J = 7.8, 1.5 Hz, 2H), 7.32 (dd, ^3J = 7.3, 2.1 Hz, 2H), 7.29 (d, ^3J = 7.9 Hz, 2H), 5.08 (d, ^3J = 3.8 Hz, 2H), 4.99–4.94 (m, 8H), 4.88 (d, ^3J = 4.0 Hz, 2H), 4.81 (d, ^3J = 6.6 Hz, 2H), 4.64 (d, ^3J = 16.2 Hz, 2H), 4.49 (d, ^3J = 16.0 Hz, 2H), 4.16 (br, 2H), 3.91 (t, ^3J = 9.3 Hz, 2H), 3.90–3.38 (m, 84H), 3.35 (d, ^3J = 16.8 Hz, 2H), 3.21 (d, ^3J = 16.8 Hz, 2H), 2.78 (br. s, 4H). FAB-MS: *m/z* 2705 (M + H).

Dimer 13. Prepared from the reaction of **1** with histamine. Yield: 41%. R_f = 0 (solvent B). ^{13}C-NMR (D_2O, CH_3CN int.): δ 174.2, 174.1, 136.5, 102.8, 102.7, 102.6, 84.2, 81.8, 81.7, 73.9, 73.6, 72.9, 72.8, 72.7, 71.8, 61.0, 60.8, 59.5, 59.2, 54.2, 41.1, 40.1, 27.3. ^{1}H-NMR (D_2O, CH_3CN int.): δ 7.61 (s, 2H), 6.83 (s, 2H), 4.98 (br, 14H), 3.95–3.43 (m, 80H), 3.40–3.10 (m, 16H), 2.69 (t, ^3J = 6.6 Hz, 4H), 2.60 (s, 4H). FAB-MS: *m/z* 2710 (M).

Dimer 14. Prepared from the reaction of **1** with benzylamine. Yield: 51%. R_f = 0.30 (solvent B). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 170.7, 170.5, 139.1, 128.1, 127.1, 126.6, 102.0, 101.8, 101.7, 83.2, 81.7, 81.5, 81.4, 81.2, 81.1, 72.8, 72.7, 72.2, 71.9, 69.4, 59.9, 59.8, 59.7, 58.1, 58.0, 52.6, 41.9, *ca.* 40.0 (embedded in the solvent signals). ^{1}H-NMR (DMSO-d_6, TMS int.): δ 8.65 (br, 2H), 7.90 (br, 2H), 7.15–7.35 (m, 10H), 5.90–5.60 (m, 28H), 4.83 (m, 14H), 4.51 (m, 12H), 4.27 (m, 4H), 3.90–3.30 (m, 92H), 2.63 (br. s, 4H). FAB-MS: *m/z* 2703 (M + H).

EDTA-bridged dimer of 6^I,6^{II}-dideoxy-6^I-amino-6^{II}-imidazolyl-β-cyclodextrin (19). 6^I-Deoxy-6^{II}-imidazolyl-6^I-*O*-(3-sulfo-2,4,6-trimethylbenzenesulfonyl)-β-cyclodextrin **16** (350 mg, 0.24 mmol) and NaN_3 (250 mg, 3.8 mmol) were added to DMF (5 ml) and the resultant mixture was stirred at 55 °C for 26 h. The reaction mixture was then slowly added to acetone (200 ml) under constant stirring and the colorless precipitates were collected by filtration. Chromatography of the precipitates on a reversed-phase Lobar column (size B) with a gradient elution from water to 30% ethanol solution afforded 6^I,6^{II}-dideoxy-6^I-azido-6^{II}-imidazolyl-β-cyclodextrin **17** (199 mg, 68%). R_f = 0.38 (solvent A). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 138.2, 128.2, 120.5, 102.4, 102.2, 102.1, 101.9, 84.0, 82.7, 81.8, 81.7, 73.5, 73.2, 73.0, 72.6, 72.3, 71.1, 69.8, 60.8, 60.0, 51.8, 47.3. ^{1}H-NMR (DMSO-d_6, TMS int.): δ 7.62 (s, 1H), 7.14 (s, 1H), 6.87 (s, 1H), 5.92–5.65 (m, 14H), 4.89–4.45 (m, 14H), 4.05 (m, 1H), 3.90–2.3 (m, 39H). FAB-MS: *m/z* 1210 (M + H). A DMF solution (10 ml) containing compound **17** (168 mg, 0.14 mmol) and triphenylphosphine (160 mg, 0.61 mmol) was stirred at rt for 2 d. Aqueous ammonia (28%, 10 ml) was then added and stirring was continued at rt for an additional 20 h. After dilution with water (50 ml), the reaction mixture was extracted with diethyl ether (50 ml × 3). The aqueous phase was concentrated and subjected to ion-exchange column chromatography (Bio-Rad AG 50W-X2, 100–200 mesh, ϕ-SO_3H type, washed with a gradient of 0 – 2.5% aqueous ammonia solution) to afford **18** (153 mg, 93%). R_f = 0 (solvent A). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 139.2, 128.0, 122.0, 102.8, 102.7, 102.6, 102.5, 102.1, 83.6, 82.4, 82.0, 81.5, 73.9, 73.7, 73.4, 73.1, 72.7, 72.6, 72.5, 72.4, 71.6, 68.3, 61.0, 48.4, 40.2. ^{1}H-NMR (DMSO-d_6, TMS int.): δ 7.66 (s, 1H), 7.18 (s, 1H), 6.99 (s, 1H), 5.15–4.85 (m, 7H), 4.8–4.45 (m, 2H, overlapped with HOD signal), 4.25 (m, 1H),

4.00–3.30 (m, 37H), 2.43 (m, 2H). FAB-MS: *m/z* 1184 (M + H), 1206 (M + Na). β-CD amine **18** (170 mg, 0.14 mmol) and EDTA dianhydride (13 mg, 0.051 mmol) were dissolved in DMF (3 ml) and the resultant solution was stirred at rt for 2 d. Water (1 ml) was then added and the reaction mixture was stirred at 60 °C for 1 h. Chromatography of the reaction mixture on a reversed-phase Lobar column (size C, gradient elution: 0–35% aqueous methanol) afforded β-cyclodextrin dimer **19** (67 mg, 50%). $R_f = 0$ (solvent A). ^{13}C-NMR (D_2O, CH_3CN int.): δ 176.0, 171.2, 138.0, 124.1, 122.9, 102.8, 102.7, 102.5, 102.4, 83.8, 83.6, 82.3, 82.1, 82.0, 74.0, 73.9, 73.6, 73.5, 73.2, 72.9, 72.8, 72.7, 71.0, 70.4, 61.5, 61.2, 61.0, 58.6, 57.9, 52.9, 49.5, 39.9. ^{1}H-NMR (D_2O, CH_3CN int.): δ 8.23 (s, 2H), 7.33 (s, 2H), 7.18 (s, 2H), 5.03 (d, $^3J = 3.5$ Hz, 2H), 4.99 (m, 10H), 4.92 (d, $^3J = 3.5$ Hz, 2H), 4.58 (overlapped with HOD, 2H), 4.34 (dd, $^3J = 14.7$, 7.2 Hz, 2H), 4.07 (d, $^3J = 8.3$ Hz, 2H), 4.96–4.42 (m, 74H), 3.41 (s, 4H), 3.32–3.26 (m, 4H), 3.14–3.06 (m, 8H). FAB-MS: *m/z* 2624 (M).

Dimer 20. This compound was prepared from dimer **19** and 2-picolylamine in a 51% yield by using the same procedures as for dimers **10–14**. $R_f = 0$ (solvent B). ^{13}C-NMR (DMSO-d_6, TMS int.): δ 171.1, 170.8, 158.6, 149.0, 138.5, 136.9, 127.9, 122.3, 121.2, 121.0, 102.5, 102.2, 83.5, 83.0, 82.5, 82.0, 81.8, 81.6, 73.3, 72.6, 72.4, 70.2, 60.8, 60.1, 58.8, 58.5, 53.0, 46.5, 44.2, *ca.* 40 (embedded in the solvent signals). ^{1}H-NMR (DMSO-d_6, TMS int.): δ 8.77 (br. s, 2H), 7.45 (d, $^3J = 3.5$ Hz, 2H), 7.77–7.64 (m, 6H), 7.23–7.15 (m, 6H), 6.80 (s, 2H), 5.90–5.63 (m, 28H), 4.93–4.74 (m, 18H), 4.62–4.36 (m, 14H), 4.17 (br, 2H), 3.92–2.94 (m, overlapped with HOD, 86H), 3.41 (br. s, 4H). FAB-MS: *m/z* 2805 (M + H).

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