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The first successful investigation into a cyclodextrin-based enzyme model as an efficient catalyst for luminol chemiluminescent reaction

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The chemiluminescence of the luminol–H_2O_2 system is found for the first time to be remarkably enhanced by the Ce(IV) complexes of cyclodextrin dimers.

A characteristic of cyclodextrins (CDs) is their ability to form inclusion compounds in aqueous solution with a large variety of chemical species ranging from organic molecules to inorganic ions. The molecules included in the CD cavity usually demonstrate chemical and physical properties quite different from those of their free forms in the bulk aqueous environment. Therefore, CDs are widely employed as molecular recognition sites to develop functional systems for diverse purposes, among which the construction of CD-based biomimetic materials[1] and molecular sensors[2] lies in the center of interest and has been witnessing great progress. Upon complex formation, molecular sensors (either themselves or their bound guests) frequently demonstrate changes in absorption/photoluminescence properties,[2] but less frequently a change in chemiluminescence (CL).[3] As enzyme mimics of oxidases, metalloporphyrins are conjugated to CD to effect oxidation of double bonds or saturated C–H bonds in a controlled manner,[1,4] and selenium is introduced in CDs to scavenge the active oxygen.[5] In this paper, we report the first example of dual CD-based metal complexes to catalyze the CL reaction of luminol.

The synthesis and structures of the dual cyclodextrins are depicted in Scheme 1. β-CD was modified with an amino group at one of the C-6 and C-3 positions by literature procedures.[6] The reactions of EDTA dianhydride with a slight excess of CD amines **3** ~ **5** were undertaken in dry DMF at room temperature and subsequent chromatography of the reaction mixtures on a reverse-phase Lobar column afforded the products **6** ~ **8** in 85, 71 and 33% yields, respectively. The structures of the dual CDs **6** ~ **8** were confirmed by FAB-MS and NMR spectra.

Cerium complexes† of the dual CDs were obtained by mixing the *aq*. solution of dual CDs with *freshly prepared aq*. solution of $Ce(NH_4)_2(NO_3)_4$, and their catalytic properties were tested on the CL reactions of luminol. To 100 μl of 0.1 M Na_2CO_3 solution 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 5s between the mixing of components and CL measurement.

Fig. 1 shows the CL decays of luminol. Catalysts are of paramount importance in aqueous luminol CL. Luminol was almost chemiluminescently mute in the absence of catalysts. None of the dual CDs, Ce(IV) ion and EDTA–Ce(IV) complex demonstrated obvious influence on the CL reaction. However, as soon as **6**–Ce(IV) complex was used, the emission of luminol was remarkably enhanced. The result is indicative of the catalytic importance of the **6**–Ce(IV) complex. The complex formation was found to take a few minutes to complete and this enabled the examination of activity–time dependence of the mixture of **6** and Ce(IV) freshly prepared from their individual solutions. The test showed the catalytic ability of the mixture increased rapidly with the mixing time and approached a

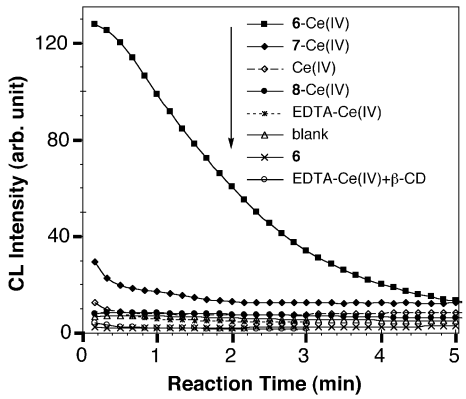
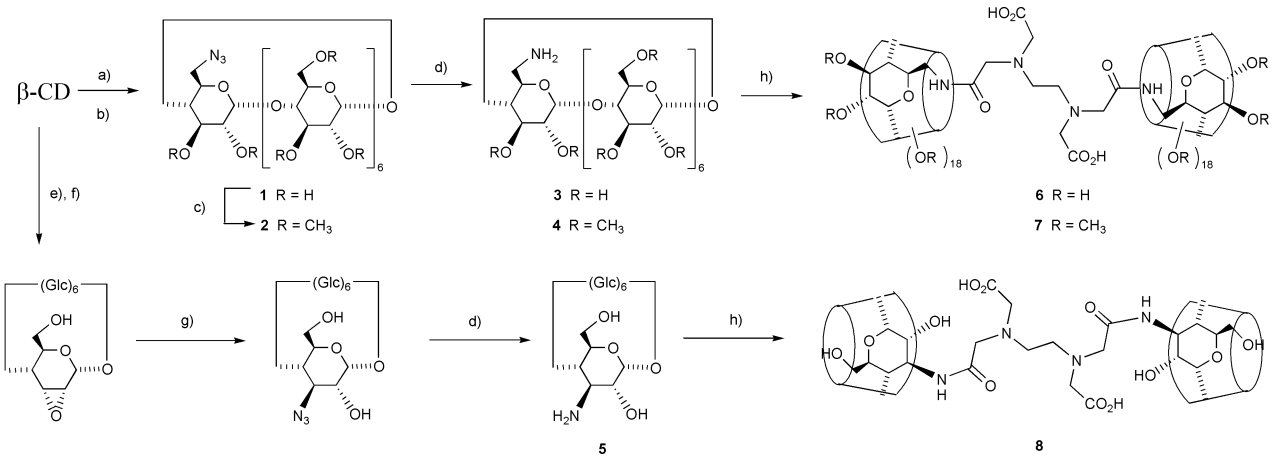


Fig. 1 The CL intensity decays in the luminol chemiluminescent reactions.



Scheme 1 The synthesis of dual cyclodextrins: (a) TsCl–pyridine; (b) NaN_3–DMF; (c) NaH–CH_3I–DMF; (d) Ph_3P–DMF; (e) 2-naphthalenesulfonyl chloride–30% *aq*. CH_3CN; (f) K_2CO_3–H_2O; (g) NaN_3–H_2O; (h) EDTA dianhydride–DMF.

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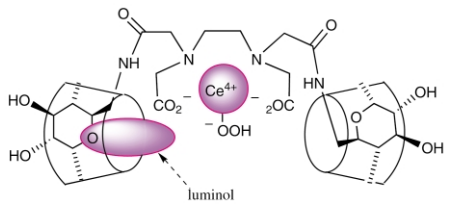

Scheme 2 Possible pre-organization of luminol and oxidant by the catalyst.

constant value about 5 min after the mixing of the individual Ce(IV) and **6** solutions, which further evidenced the catalytic importance of complex formation.

Many metal cations are well-known to catalyze this reaction, increasing the light emission or at least speeding up the oxidation to produce the emitter and therefore the onset of light production.[7] It has also been reported that the binding of luminol to hydrophobic regions could strongly enhance the luminol CL.[8] 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 one single molecule just as most metalloenzymes do. It is therefore the covalent conjugation and cooperation of β-CD and EDTA-Ce(IV) components that are essential for the catalytic ability since neither the component individuals nor their mechanical combination resulted in obvious CL changes.

Based on the above results we tentatively propose a pre-organization of the two reactants by the catalyst (Scheme 2) for the **6**–Ce(IV) catalyzed chemiluminescent reaction: the Ce(IV) center binds the HOO^- while the CD hydrophobic cavity brings luminol close to the bound HOO^-, thus the local concentrations of both luminol and oxidant are greatly increased. The CL is switched on only when the metal center and the hydrophobic cavity can efficiently cooperate, which means the geometry of the dual cyclodextrin would be very important, and this was proved to be the case. When the dual CD **8** was used instead of **6**, no enhancement of luminol CL was observed. It is worthy of note that compounds **8** and **6** differ only in the positions at which the EDTA linkage is attached to CD moieties. Dual CD **8** has CD cavities of the same shape as, but opposite arrangement to those of compound **6**. CL measurements indicated that this difference in cavity arrangement in **8** resulted a dramatic loss of catalytic ability. The remarkable difference in catalytic ability of the two dual CDs can be rationalized as follows. The secondary side of CD is less hydrophobic but more acidic. Under the experimental condition (pH ≈ 11.5), it will ionize and become even worse for 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, dual CD **6** is expected to locate luminol towards the center of the molecule where the oxidant is bound, whereas **8** directs luminol outwards to either terminals of the molecule. In addition, the ionized secondary OH groups of **8** may compete with HOO^- in coordinating to the Ce(IV) on the same side. Breslow demonstrated that in heptakis(6-methylamino)–CD, the methyl groups were inserted inwards to the CD cavity forming a floor.[9] It is reasonable to deduce that permethylation of the dual CD **6** would inverse the orientation of luminol, that is, the luminol molecule would be accommodated at the secondary side instead

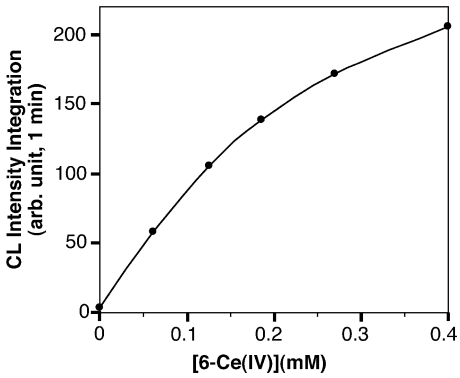

Fig. 2 CL emission *versus* catalyst concentrations.

of the primary side near the Ce(IV) center. Indeed the permethylated dual CD **7**–Ce(IV) showed a catalytic activity only one fourth that of **6**–Ce(IV).

We also examined the influence of catalyst concentration on the CL intensity. Upon varying [**6**–Ce(IV)] from 0 to 0.4 mM, the CL intensity increased proportionally at first and then showed increasing downward deviation (Fig. 2).

In conclusion, the present research demonstrated the first enzyme model that has both substrate-binding and catalytic sites to catalyze the luminol chemiluminescent reaction. The cooperation of the hydrophobic binding site and the catalytic Ce(IV) center was proved to be of pivotal importance for getting high efficacy in amplifying the luminol CL. The idea of pre-organizing the chemiluminescent 'fuels' and oxidants by enzyme models may offer many opportunities to improve the CL as well as a promising approach to develop high efficacy but low cost CL systems.

Notes and references

† Freshly prepared Ce solution should be used to prepare the Ce complexes, otherwise the activity will be greatly lost. The Ce complexes were stable, and did not show obvious loss of activity even after being stored for a few weeks. Even so, the complex solutions were all freshly prepared and used within one hour.

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