行政院國家科學委員會專題研究計畫　成果報告

包絡物增強 luminol 化學發光及應用於客體分子偵測之研究

計畫類別：個別型計畫
計畫編號：NSC93-2113-M-002-036-
執行期間：93 年 08 月 01 日至 94 年 10 月 31 日
執行單位：國立臺灣大學化學系暨研究所

計畫主持人：林萬寅

報告類型：精簡報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 95 年 1 月 23 日

行政院國家科學委員會補助專題研究計畫 ☑ **成 果 報 告**
□期中進度報告

利用包絡物增強化學發光及其應用之研究

計畫類別：☑ **個別型計畫**　　□ **整合型計畫**
計畫編號：NSC 93－M－2113－002 －036 －
執行期間： 93 **年** 8 **月** 1 **日至** 94 **年** 10 **月** 31 **日**

計畫主持人：林萬寅
共同主持人：
計畫參與人員：

成果報告類型(依經費核定清單規定繳交)：☑**精簡報告**　□**完整報告**

本成果報告包括以下應繳交之附件：
□**赴國外出差或研習心得報告一份**
□**赴大陸地區出差或研習心得報告一份**
□**出席國際學術會議心得報告及發表之論文各一份**
□**國際合作研究計畫國外研究報告書一份**

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：

中　華　民　國 95 **年** 1 **月** 23 **日**

中文摘要：

β-環糊精可以顯著增強 luminol 在鹼性溶液中被氧化劑如 $KMnO_4$、KIO_4、NaClO、N-bromosuccinimide 氧化所產生之化學發光達一至二個數量級以上。環糊精可以將反應中間體或放光物質包在其空腔中，具有穩定這些物質及隔離自由基或螢光消滅劑，而達到顯著增強化學發光的功效。我們也詳細探討了 pH 值、氧化劑、luminol、增強劑濃度對化學發光的影響。這些系統可用於檢驗參與或影響化學發光反應之物質，包括化學發光試劑、增強劑、抑制劑(如抗氧化劑、胺基酸、類黃酮等)。

關鍵詞：化學發光、luminol、環糊精

Abstract

The chemiluminescence (CL) from the oxidation of luminol with several oxidants (e.g., $KMnO_4$, N-bromosuccinimide, KIO_4, NaClO) in alkaline solution was enhanced by 1~2 orders of magnitude upon addition of β-cyclodextrin (β-CD). Inclusion of reaction intermediates and/or the CL-emitter in the β-CD cavity help stabilize them and shield them from external radical scavengers or fluorescence quenchers, leading to dramatic enhancement in CL emission. The effects of pH and concentrations of oxidants, luminol and enhancer on CL have been systematically studied. These enhanced CL systems were employed to determine the substances that participate or influence the CL reactions, including CL reagents, enhancers, and inhibitors (e.g., antioxidants, amino acids, flavonoids).

Keywords: Chemiluminescence, luminol, cyclodextrin

計劃緣由與目的：

Chemiluminescence (CL) detection is a very attractive analytical tool owing to its superb sensitivity, wide dynamic range, simple instrumentation, low cost, fast analysis, and easy automation. In the past few years, we have been interested in the enhancement of CL emission from the oxidation of luminal using a variety of catalysts and oxidants. One of the very effective catalyst for the oxidation of luminol is microperoxidase (MP), which is obtained from proteolytic digestion of cytochrome c.[1,2] Intense CL emission was observed for the oxidation of luminol with H_2O_2 or m-chloroperoxybenzoic acid catalyzed by Fe- or Mn-MP.[3] In addition, some enhancers, such as Na_2CO_3, Tris,

guanidine hydrochloride, are capable of increasing their CL emission by 1-2 orders of magnitude, depending on the catalysts and oxidants used.[4-7]

In this project, we have found that the use of β-cyclodextrin (β-CD) caused a dramatic enhancement for the CL emission from the oxidation of luminol with a variety of oxidants, including $KMnO_4$, KIO_4, NaClO, N-bromosuccinimide (NBS). We have investigated the effects of pH and concentrations of oxidants, luminol and enhancer on the CL emission. We also studied the potential applications of these enhanced CL systems.

結果與討論：

CL for the oxidation of luminol with $KMnO_4$

CL emission involving the oxidation of CL reagents with $KMnO_4$ is usually carried out in an acidic solution. In alkaline solution, extremely weak CL emission is normally observed. Fig. 1 shows the CL emission obtained by a flow injection analysis (FIA) manifold for the oxidation of luminol with $KMnO_4$ at pH 10.0 in the presence of various concentrations of β-CD.

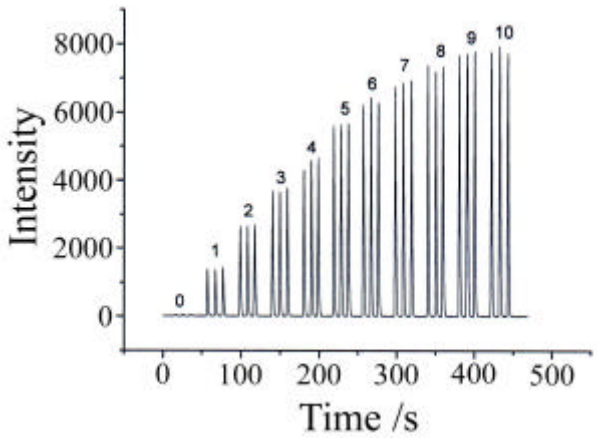

Fig. 1. FIA-CL emission for the oxidation of 100 μM luminol with 100 μM $KMnO_4$ (pH 10.0) at various concentration of β-CD. [β-CD] in mM was indicated in the figure.

Extremely weak CL emission was observed without β-CD. The CL emission was dramatically enhanced by the addition of β-CD and an enhancement of 250 folds was achieved with 5 mM β-CD. The use of α-CD, γ-CD or surfactants (e.g., sodium lauryl sulfate or cetyltrimethylammonium bromide) causes only a slight enhancement (less than 2 folds) of the CL emission. The results suggest that cavity size plays an important role in CL enhancement. Thus the overall CL efficiency was greatly increased through the inclusion of reaction intermediates and/or CL emitter in the β-CD cavity.

The CL intensity increases linearly over the concentration range of 10-100 μM for both luminol and $KMnO_4$ (data not shown). The enhanced CL system can be employed to determine a variety of substances. Fig. 2

shows the effect of various amino acids (100 μM each) on the CL emission for the luminol/$KMnO_4$/β-CD system. The conditions in Fig. 1 were used throughout this study.

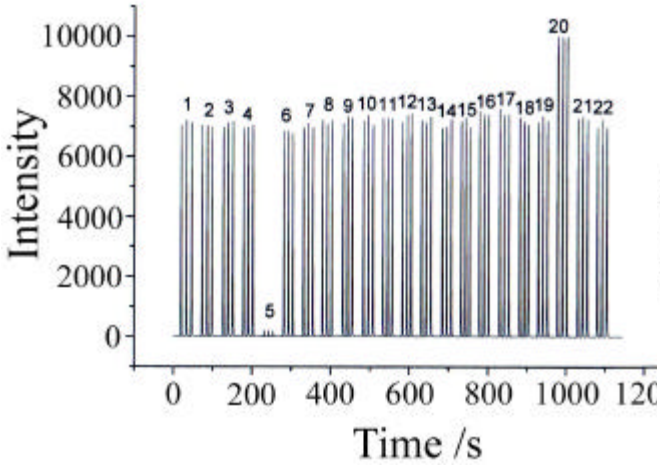

Fig. 2. FIA-CL emission for luminal/$KMnO_4$/β-CD at pH 10.0 in the presence of 100 μM of various amino acids. 1. None 2. Asn 3. Leu 4. Phe 5. Cys 6. Cystine 7. Met 8. Glu 9. Gln 10. Lys 11. His 12. Val 14. Ile 14. Ser 15. Asp 16. Arg 17. Pro 18. Thr 19. Ala 20. Tyr 21. Gly 22. Trp

It was found that Cys inhibited the CL emission, while Tyr enhanced it. The rest of amino acids showed essentially no effect on CL emission at 100 μM level. The CL system can be used to determine Tyr (0-10 μM) or Cys (0-20 μM) as demonstrated in Fig. 3. Antioxidants such as trolox (a water-soluble vitamin E equivalent), L-ascorbic acid, and 3-t-butyl-4-hydroxyanisole (BHA) (at 1~ 20 μM level) inhibit the CL emission significantly, allowing their quantification (data not shown).

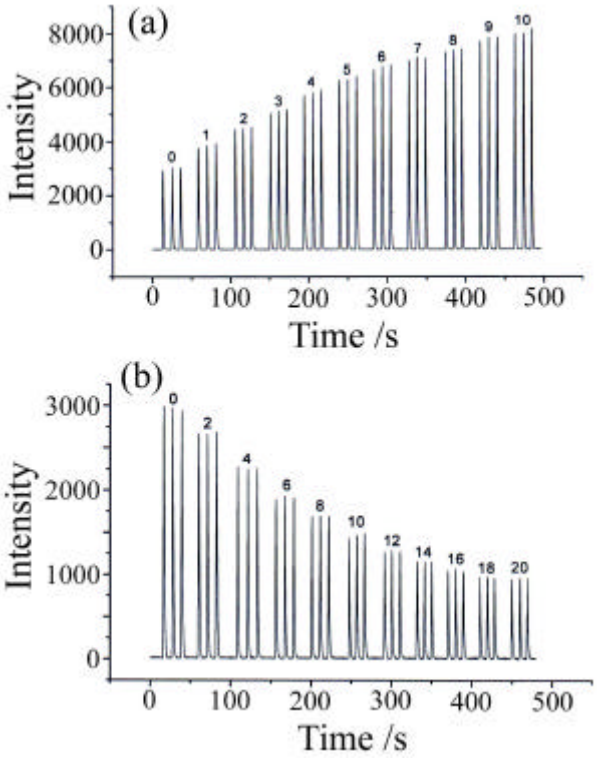

Fig. 3. FIA-CL emission for luminol/$KMnO_4$/β-CD at pH 10.0 in the presence of (a) Tyr and (b) Cys. The concentration in mM was indicated in the figure.

CL for the oxidation of luminol with KIO_4

β-CD is also a very effective enhancer for the CL emission from the oxidation of luminol with KIO_4 as demonstrated in Fig 4. In the absence of β-CD, essentially no CL emission was observed for the luminol/KIO_4 system. Tremendous enhancement in CL emission was observed upon addition of β-CD. The use of α-CD and γ-CD exhibited much less CL enhancement (about 15% of β-CD) for the KIO_4/luminol system. It suggests the necessity of using CD of suitable cavity size for efficient complexation with the guest molecules to induce a substantial CL enhancement.

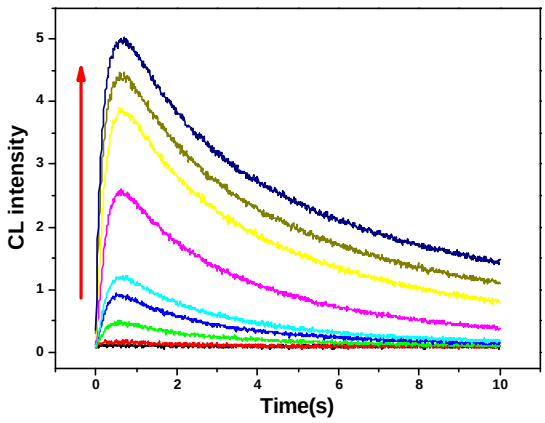

Fig. 4. Stopped-flow CL intensity versus time profiles for the oxidation of 50 μM luminol with 0.2 mM KIO_4 at pH 12.0 in the presence of various concentrations of β-CD indicated in the figure (0-5 mM; from bottom to top).

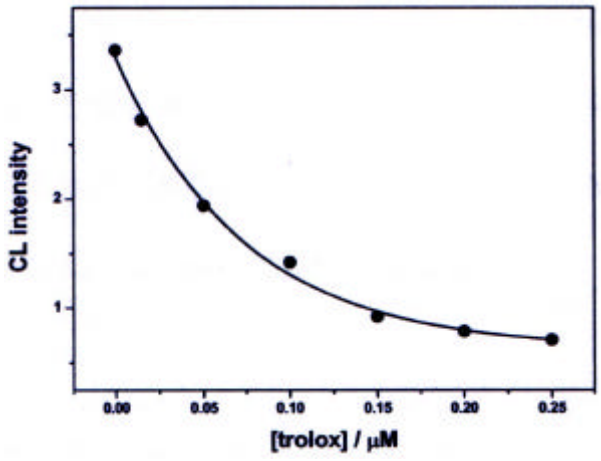

Fig. 5. The CL intensity of the luminol/KIO_4/β-CD system in the presence of various concentrations of trolox. The experimental conditions were the same as in Fig. 4.

The CL intensity increases with increasing concentrations of KIO_4 (0.05 ~ 0.3 mM) and luminol (15 ~ 75 μM). Moreover, the CL intensity increased ~10 folds as the temperature was raised from 15 to 35°C (data not shown). This result might be due to an accelerated rate of CL reaction at high temperature, leading to an increased yield of light emitter and hence CL emission.

The CL system of luminol/$KMnO_4$/β-CD was found to be very sensitive for determi ning low level (0.01 ~ 0.25 μM) of trolox as shown in Fig. 5. It is also applicable to the detection of BHA and a variety of flavonoids (Table 1).

Table 1. IC50[a] and linear ranges of antioxidants

Antioxidants	Linear range (M)	IC_{50} (μM)
Trolox	1.5×10^{-8} ~ 2.5×10^{-7}	0.051
BHA	5×10^{-8} ~ 6.5×10^{-7}	0.129
3,7-dihydroxyflavone	5×10^{-8} ~ 7×10^{-7}	0.207
3,6-dihydroxyflavone	5×10^{-8} ~ 1×10^{-6}	0.212
3,3'-dihydroxyflavone	1×10^{-6} ~ 1×10^{-5}	4.384
3-hydroxyflavone	2.5×10^{-6} ~ 2.5×10^{-5}	6.458

[a] Concentration of antioxidant for 50% inhibition of CL emission

CL for the oxidation of luminol with NaClO

The presence of various CDs exhibits quite different effects on the CL emission from the oxidation of luminol with NaClO as shown in Fig. 5. Only β-CD shows substantial enhancement in CL emission. α-CD has essentially no effect, while γ-CD and dimethyl-β-CD inhibit it. The results support the requirement for comparable sizes of host and guest molecules to facilitate complexation and hence CL enhancement.

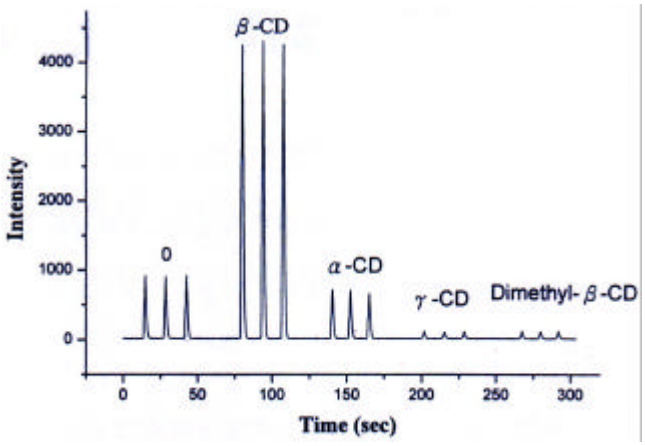

Fig. 6. FIA-CL emission for the oxidation of luminol (100 μM) with NaClO (50 μM) at pH 9.5 in the presence of 5 mM of various CDs.

The CL emission of the system luminol/NaClO/β-CD is inhibited by S-containing amino acids (Cys and Met) as shown in Fig. 7.

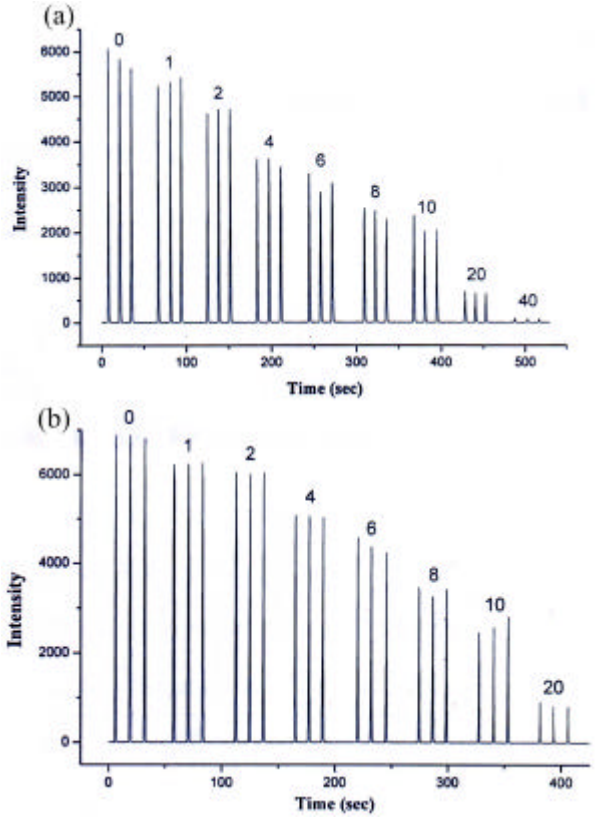

Fig. 6. FIA-CL emission for the luminal/NaClO/β-CD system in the presence of various concentration (in mM and was indicated in the figure) of (a) Cys and (b) Met.

It allows the determination of Cys and Met at 1-20 μM level. Other amino acids have no effect on the CL emission for this system. Other antioxidants such as glutathione (1-20 μM), hydroquinone (1-50 μM), quinic acid (1-10 μM), sesamol (0.1-3.0 μM), trolox (1-30 μM) have also been determined successfully by this enhanced CL system (data not shown).

CL for the oxidation of luminol with NBS

b-CD exhibited a dramatic enhancement in CL emission for the oxidation of luminal (10 mM) with N-bromosuccinimide (12.5 mM) at pH 10.5 as demonstrated in Fig. 7.

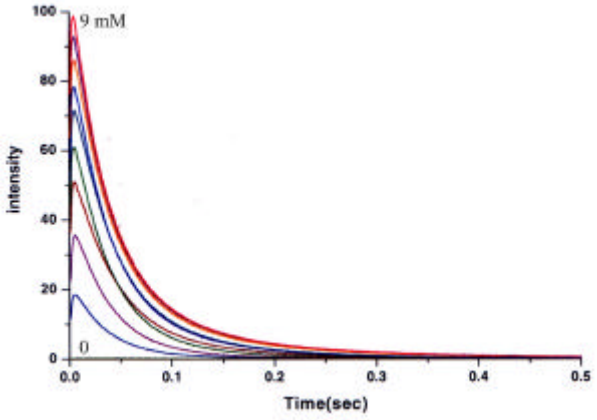

Fig. 7. Stopped-flow CL intensity versus time profiles for the oxidation of 10 μM luminol with 12.5 μM NBS at pH 10.5 in the presence of various concentrations (bottom to top: 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 mM) of β-CD.

The peak CL intensity of this system increased by 74 folds upon addition of 9 mM β-CD. Moreover, the CL emission lasts only 0.5 s, revealing the necessity for a special technique such as a stopped-flow instrument to record the short-lived emission.

The CL intensity increases linearly with

increasing concentration of luminol over the range 10-150 μM. The CL intensity also increases as the temperature increases from 15 to 35°C, similar to the observation for the luminol/KIO_4/β-CD system.

The CL emission of luminol/NBS/β-CD is inhibited by Cys and Met. The effect of Cys on the CL emission is shown in Fig. 8.

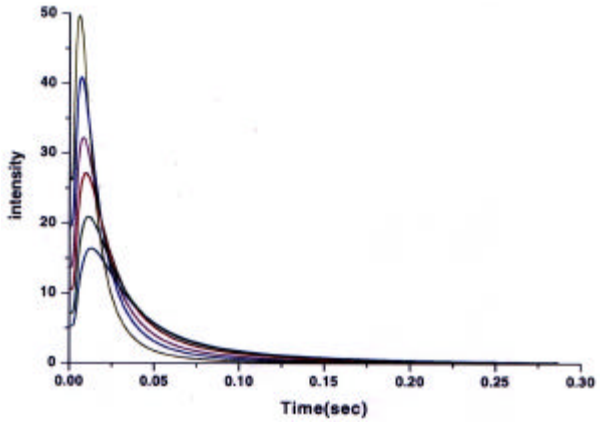

Fig. 8. Stopped-flow CL intensity versus time profiles for the luminol/NBS/β-CD system in the presence of various concentrations (top to bottom: 0, 10, 20, 30, 40, 50 μM) of Cys. .

Interestingly, L-ascorbic acid further enhanced the CL emission of the luminol/NBS/β-CD system as illustrated in Fig. 9. The CL intensity increased linearly with the increasing concentration of L-ascorbic acid over the range of 0-35 μM, allowing its quantification. Trolox and BHA inhibit the CL emission linearly over the concentration range of 0-60 μM (data not shown)

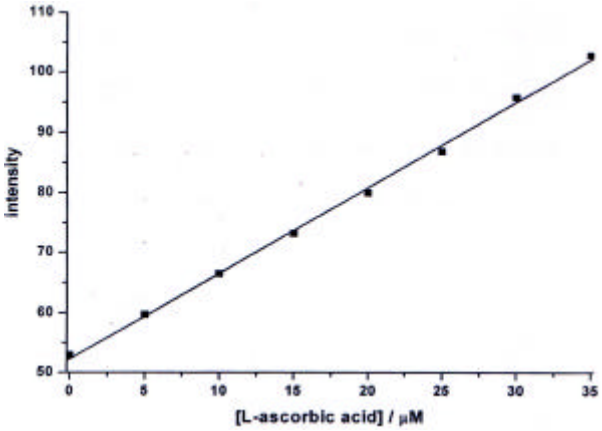

Fig. 8. Dependence of the CL intensity for the system luminol/NBS/β-CD on the concentration of L-ascorbic acid. .

Conclusion

We have found that β-cyclodextrin is capable of enhancing the CL emission from the oxidation of luminol with $KMnO_4$, KIO_4, NaClO, and N-bromosuccinide in alkaline solution by 1~2 orders of magnitude. The inclusion of reaction intermediates and/or CL emitter in the β-CD cavity plays a major role for the CL enhancement. These enhanced CL systems were employed successfully to determine a variety of substances including Cys, Met, Tyr, trolox, L-ascorbic acid, BHA, and other antioxidants.

References:

1. Yeh, H. –C.; J. Wang, –S.; Su, Y. O. Lin, W. –Y. *J. Biol. Inorg. Chem.* **2001**, *6*, 770-777.
2. Yeh, H. –C.; Yu, J. –H.; J. Wang, –S.; Chen, S. –T.; Su, Y. O. Lin, W. –Y. *J. Biol.*

Inorg. Chem. **2002**, *7*, 113-119.

3. Yeh, H. –C.; Lin, W. –Y. *Anal. Chim. Acta,* **2001**, *442*, 71-77.
4. Yeh, H. –C.; Lin, W. –Y. *Anal. Bioanal. Chem.* **2002**, *372*, 525-531.
5. Yeh, H. –C.; Lin, W. –Y. *Talanta,* **2003,** *59*, 1029-1038.
6. Yeh, H. –C.; Lin, W. –Y. *J. Chin. Chem. Soc.* **2003**, *50*, 81-88.
7. Yeh, H. –C.; Hsu, W. –T.; Lin. W. –Y. *J. Chin. Chem. Soc.* **2005**, *52*, 657-664.