

行政院國家科學委員會專題研究計畫 期中進度報告

微過氧化酵素催化 luminol 之化學發光、增強劑之開發及應用(2/3)

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計畫主持人：林萬寅

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# 行政院國家科委員會專題研究期中報告(II)

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計劃主持人：林萬寅 執行單位：國立台灣大學化學系

## 中文摘要：

鐵-及錳-微過氧化酵素(MP)能有效的催化 luminol 之化學發光。催化能力錳-MP 較鐵-MP 好，氧化力 mCPBA (*m*-chloroperoxybenzoic acid) 比 H<sub>2</sub>O<sub>2</sub> 強。pH 10.0 時，Guanidine hydrochloride 能增強鐵-MP/mCPBA、錳-MP/H<sub>2</sub>O<sub>2</sub> 及錳-MP/mCPBA 之 luminol 化學發光分別為 60、165 及 26 倍；Na<sub>2</sub>CO<sub>3</sub> 能增強鐵-MP/H<sub>2</sub>O<sub>2</sub> 之 luminol 化學發光 25 倍。我們也詳細探討了催化劑、氧化劑、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如抗氧化劑。

關鍵詞：微過氧化酵素、化學發光、增強劑

## Abstract

Fe- and Mn-microperoxidase (MP) are very effective catalysts for the chemiluminescence (CL) reaction of luminol. Mn-MP is better than Fe-MP as a catalyst, while mCPBA is better than H<sub>2</sub>O<sub>2</sub> as an oxidant for the CL reaction of luminol. Guanidine hydrochloride enhances the CL intensity of luminol for Fe-MP/mCPBA, Mn-MP/H<sub>2</sub>O<sub>2</sub>, and Mn-MP/mCPBA at pH 10.0 by 60-, 165- and 26-folds, respectively; while Na<sub>2</sub>CO<sub>3</sub> increases the CL intensity of

Fe-MP/H<sub>2</sub>O<sub>2</sub>/luminol by 25 folds. The effects of the concentrations of catalyst, oxidant and enhancer on CL have been systematically studied. These systems can be employed to detect any substance that participates or influences the CL reactions (e.g., antioxidants).

Keywords: Microperoxidase, Chemiluminescence, Enhancer

## 計劃緣由與目的：

Microperoxidase (MP) exhibits peroxidase, P-450, and chloroperoxidase activities. We have investigated the kinetics and substrate specificity for the oxidation of a variety of substrates with H<sub>2</sub>O<sub>2</sub> catalyzed by Fe-MP<sup>1</sup> and Mn-MP<sup>2</sup>. Intense chemiluminescence (CL) was observed for the oxidation of luminol with H<sub>2</sub>O<sub>2</sub> or mCPBA catalyzed by Fe- or Mn-MP. Moreover, some enhancers are found to increase the CL intensity by orders of magnitude. In this project, we have investigated these CL systems in terms of the effects of the concentrations of catalyst, oxidant, enhancer, and the pH on the CL emission and their potential applications.

## 結果與討論：

### *CL of MP/luminol/H<sub>2</sub>O<sub>2</sub> or mCPBA*

We have studied the CL from the oxidation of luminol with H<sub>2</sub>O<sub>2</sub> or mCPBA catalyzed by Fe- or Mn-MP8.<sup>3</sup> The CL emission involving Mn-MP8 is 2-3 times as intense as that of Fe-MP8, whereas using mCPBA in place of H<sub>2</sub>O<sub>2</sub> as the oxidant increases CL by 12 ~ 18 folds at pH 12 as shown in Fig. 1..

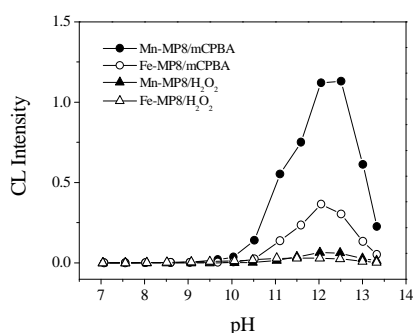


Fig. 1. pH dependence of the peak CL intensity for the oxidation of luminol with H<sub>2</sub>O<sub>2</sub> or mCPBA catalyzed by Fe- or Mn-MP8.

The acceleration of the CL cycle plays a major role in the CL enhancement as illustrated in Fig. 2. The use of mCPBA

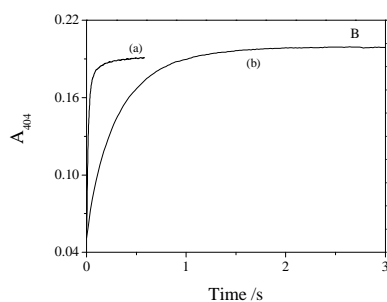


Fig. 2. Formation of the intermediate of Mn-MP8 by adding (a) mCPBA (b) H<sub>2</sub>O<sub>2</sub>.

in place of H<sub>2</sub>O<sub>2</sub> increases the rate of the formation of intermediate (monitored at 404 nm) by ~ 10 folds.

### *Enhancers for Fe-MP/luminol/mCPBA*

We have found that the addition of guanidine hydrochloride (GdnHCl), Na<sub>2</sub>CO<sub>3</sub>, or NaCl dramatically enhances the CL emission of the Fe-MP8/luminol/mCPBA system.<sup>4</sup> As shown in Fig. 3, the addition of GdnHCl increases the CL intensity significantly, whereas the CL duration decreases progressively.

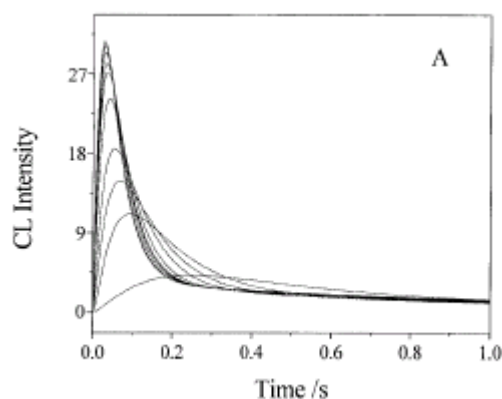


Fig. 3. CL intensity vs. time profiles for Fe-MP8/luminol/mCPBA at various conc. of guanidine hydrochloride (bottom to top: 0, 0.5, 0.1, 0.15, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0 and 2.5 M).

The enhancements in CL for GdnHCl, Na<sub>2</sub>CO<sub>3</sub>, and NaCl at pH 10.0 are 60-, 49-, and 37-folds. The CL enhancement is due primarily to the acceleration of the CL cycle.

### *Enhancers for Fe-MP/luminol/H<sub>2</sub>O<sub>2</sub>*

The presence of Na<sub>2</sub>CO<sub>3</sub> and Tris increases the CL emission significantly (Fig. 4).<sup>5</sup> Maximum enhancements of 19.0 and 9.2 at pH 10.0 were achieved

for 0.3 M  $\text{Na}_2\text{CO}_3$  and 0.1 M Tris, respectively. Acceleration of the CL cycle and the formation of carbonate and Tris radical play important roles in the CL enhancement.

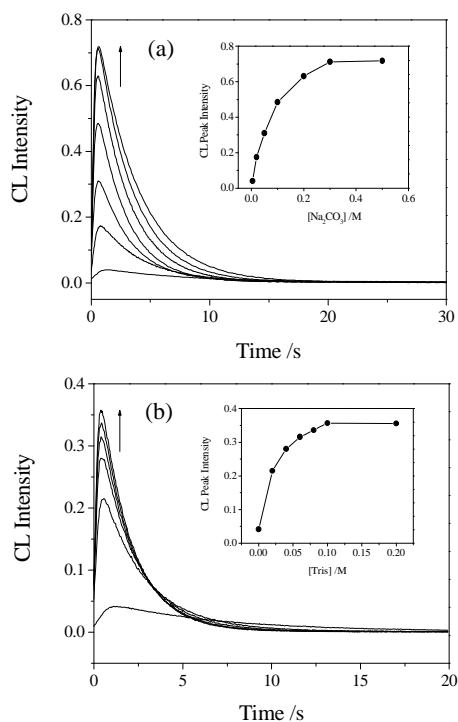


Fig. 4. Effects of the conc. of (a)  $\text{Na}_2\text{CO}_3$  (0-0.3 M) and (b) Tris (0-0.1 M) on the CL intensity vs. time profiles for Fe-MP8/luminol/ $\text{H}_2\text{O}_2$ .

As an illustration of the application of the enhanced CL system, the detection of ascorbic acid was demonstrated in Fig. 5. The presence of increasing amount of ascorbic acid dramatically delayed (Fig. 5, inset) and diminished the CL emission, allowing the determination of its concentration using the time delay and the intensity reduction.

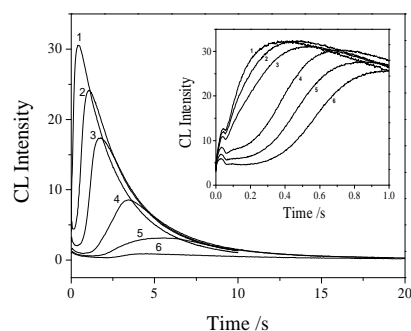


Fig. 5. The CL intensity vs. time profiles for Fe-MP8/luminol/ $\text{H}_2\text{O}_2/\text{Na}_2\text{CO}_3$  in the presence of various conc. of ascorbic acid. Inset: 0-1  $\mu\text{M}$ ; 1-6: 0, 1.0, 2.5, 5.0, 7.5, 10.0  $\mu\text{M}$ .

#### Enhancers for Mn-MP/luminol/ $\text{H}_2\text{O}_2$

The addition of  $\text{Na}_2\text{CO}_3$  (0.5 M) and GdnHCl (3 M) enhances the CL emission of Mn-MP8/luminol/ $\text{H}_2\text{O}_2$  to different degree at various pH as shown in Fig. 6.<sup>6</sup> The maximum enhancements of 165- and 56-folds were obtained at pH 10.0 for 0.5 M  $\text{Na}_2\text{CO}_3$  and 3 M GdnHCl, respectively. The CL signal covers several orders of magnitude over a wide conc. range of luminol and  $\text{H}_2\text{O}_2$  (data not shown).

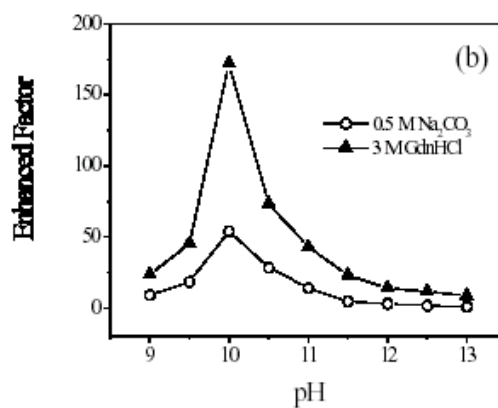


Fig. 6. Enhanced factors for the CL emission of Mn-MP8/luminol/ $\text{H}_2\text{O}_2$  in the presence of (○) 0.5 M  $\text{Na}_2\text{CO}_3$  and (▲) 3 M GdnHCl.

The CL emission spectrum exhibits only a single band at 425 nm, corresponding to the emission of the excited 3-aminophthalate (Fig. 7). Therefore, the enhancement is due to the accelerated and increased production of the luminol radical and hence product.

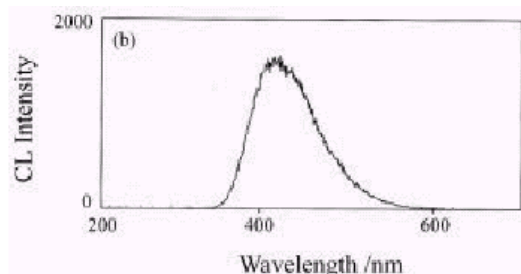


Fig. 7. The CL emission spectrum of Mn-MP8/luminol/H<sub>2</sub>O<sub>2</sub>/Na<sub>2</sub>CO<sub>3</sub>.

#### Enhancers for Mn-MP/luminol/mCPBA

The addition of Na<sub>2</sub>CO<sub>3</sub>, GdnHCl, NaCl, and Tris to Mn-MP8/luminol/mCPBA causes an enhancement in CL emission to various degrees at different pH as illustrated in Fig. 8.<sup>7</sup>

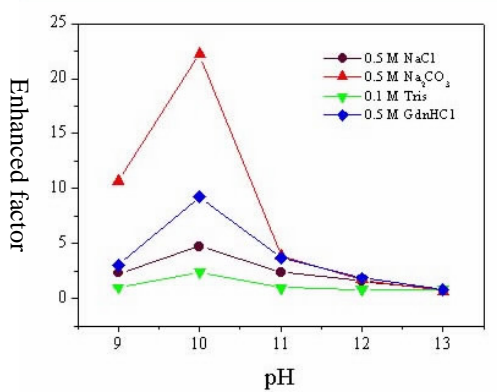


Fig. 8. Enhanced factors for the CL emission of Mn-MP8/luminol/mCPBA in the presence of ( ) 0.5 M Na<sub>2</sub>CO<sub>3</sub> and ( ) 0.5 M GdnHCl, ( ) 0.5 M NaCl, ( ) 0.1 M Tris.

Maximum enhancement in CL of

22-folds was achieved for 0.5 M Na<sub>2</sub>CO<sub>3</sub> at pH 10.0. These CL systems can be employed as very sensitive CL assays for substances that participate or influence the CL reaction.

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