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ORIGINAL PAPER

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Enhanced chemiluminescence for the oxidation of luminol with *m*-chloroperoxybenzoic acid catalyzed by microperoxidase 8

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Abstract The use of *m*-chloroperoxybenzoic acid (*m*CPBA) in stead of hydrogen peroxide causes an increase in chemiluminescence (CL) of luminol oxidation catalyzed by microperoxidase 8 (MP8) by an order of magnitude. The accelerated formation of an intermediate plays a major role in the CL enhancement, which also leads to a significant reduction in CL duration. The presence of guanidine hydrochloride, sodium carbonate, or sodium chloride further increases the CL emission drastically. The CL emission enhancement is strongly pH dependent. The enormous enhancement of the CL signal is due to an accelerated CL cycle and an improved CL efficiency in the presence of the enhancer. The CL signal covers several orders of magnitude over a wide range of concentrations of luminol and *m*CPBA. The intense CL of MP8-luminol-*m*CPBA in the presence of the enhancer will have great potential for extremely sensitive CL assays.

Keywords Chemiluminescence · Stopped-flow · Microperoxidase 8 · *m*-Chloroperoxybenzoic acid · Enhancer

Abbreviations *CL* chemiluminescence · *MP8* microperoxidase 8 · *mCPBA m*-chloroperoxybenzoic acid · *GdnHCl* guanidine hydrochloride

Introduction

Chemiluminescence (CL) based on the peroxidase (e.g., horseradish peroxidase [1], lactoperoxidase [2], myeloperoxidase [3]) catalyzed oxidation of luminol by hydrogen peroxide has been well documented. However, the applications of the CL involving peroxidases are usually limited by the low activity and stability at extreme pH and in the presence of organic solvents due to the denaturation of the proteins. Microperoxidase (MP) is an attractive catalyst for peroxidase activity; MPs contain a covalently linked oligopeptide (e.g., with 8 amino acids for MP8) and do not require the maintenance of the native peptide structure to retain proper functionality. The MP-catalyzed oxidation of luminol by hydrogen peroxide has been employed for the determination of a variety of substances, including peroxides [1], antioxidants [4], and catecholamines [5]. In this paper, the stopped-flow technique was applied, using *m*-chloroperoxybenzoic acid (*m*CPBA) as the oxidant, to the MP8-luminol system which led to enhancement of the CL emission by more than an order of magnitude in comparison with H_2O_2. Moreover, the presence of enhancers such as guanidine hydrochloride (GdnHCl), sodium carbonate, or sodium chloride dramatically increases the CL emission of the MP8-luminol-*m*CPBA system. We also investigated the effects of pH, concentrations of oxidant, luminol, and enhancers on the CL emission involving MP8 and *m*CPBA. Finally, the mechanism for the CL enhancement has been discussed.

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Experimental

Materials

Horse heart cytochrome *c*, pepsin, trypsin, sodium phosphate, sodium hydrogen phosphate, luminol (5-amino-2,3-dihydrophthalazine-1,4-dione), and guanidine hydrochloride were purchased from Sigma (St. Louis, MO, USA). Sodium carbonate, uric acid, and *m*-chloroperoxybenzoic acid (*m*CPBA) were obtained from Acros (Geel, Belgium). Sodium chloride was purchased from Merck (Darmstadt, Germany). All reagents were of the highest grade of purity. Ultra high purity deionized water was obtained from a Milli-Q purification system (18.2 MΩ cm^{-1}, Millipore, Tokyo, Japan). The stock solution of hydrogen peroxide (Fluka, Seelze, Germany) was prepared by volumetric dilution of 30% (w/w) H_2O_2 by pH 7.0 phosphate buffer. The concentration of H_2O_2 was determined daily by measuring the absorbance at 240 nm on a Hitachi U-3210 spectrophotometer (Hitachi, Tokyo, Japan) using ε_{240}=39.4 M^{-1} cm^{-1} [6]. MP8 was prepared by the proteolytic digestion of cytochrome *c* with pepsin and trypsin according to the procedure in the literature [7]. The concentrations of MP8 were also determined spectrophotometrically using $\varepsilon_{397}=1.57\times10^5$ M^{-1} cm^{-1} at pH 7.0 [8].

The stock solutions of luminol and *m*CPBA were prepared by dissolving the precisely weighed substance in 5 mM phosphate buffer at the desired pH.

Stopped-flow CL and kinetic measurements

All the stopped-flow experiments (CL, change in the absorbance, and time-resolved absorption spectra) were performed on a HITECH SF-61 DX2 stopped-flow spectrofluorimeter (Hitech Scientific, Salisbury, UK) similar to that described previously [9]. The CL experiments were performed in the fluorescence mode with the light source switched off. The MP8 solution was stored in one syringe while the luminol-oxidant mixture was stored in the other. Both solutions contained an appropriate amount of NaCl, Na_2CO_3, or GdnHCl. The CL intensity versus time profile was recorded after 1:1 mixing of the solutions. All solutions in the CL measurements (before mixing) were prepared in 5 mM phosphate buffer and the pH values were adjusted with saturated NaOH or concentrated HCl using a pH meter (Model 6071, Jenco Electronics Ltd, Taipei, Taiwan). The time-resolved absorption spectra were recorded with a photodiode array detector. The formation rates of the intermediate were measured by continuously monitoring the change in absorbance at 417 nm after mixing the MP8 and the oxidant in two separate syringes. The experiments were carried out in the absorption mode with the PMT voltage set at 200 V. For measuring the rate (or the CL intensity) of the reaction of the intermediate with luminol, MP8, and *m*CPBA, solutions (with or without enhancers) stored in two separate syringes were allowed to mix in a mixer channel. After an appropriate delay to attain a maximum amount of intermediate, the mixture was then further mixed with luminol stored in a third syringe and the change in absorbance at 417 nm (or CL intensity) was recorded continuously. All solutions in the kinetic studies were prepared in 5 mM phosphate buffer (adjusted to an appropriate pH) in the absence or presence of 3.0 M NaCl, 1.0 M Na_2CO_3, or 2.5 M GdnHCl. The built-in software of the stopped-flow instrument allows multiple exponential fitting of the traces. All the stopped-flow experiments were carried out at 25 °C. Six replicate measurements were performed for each CL experiment and ten replicate measurements for each kinetic analysis.

Measurement of the CL emission spectrum

The emission spectrum was recorded using a continuous-flow manifold in conjuction with a fluorimeter as described previously [10]. One of the flow solutions contained 5.0 μM MP8 and 100 μM luminol, the other contained 5.0 μM *m*CPBA. Both solutions also contained 2.5 M GdnHCl (or 1.0 M Na_2CO_3 or 3.0 M NaCl) in 5 mM phosphate buffer at pH 12.0.

Results and discussion

Stopped-flow CL profiles of MP8-luminol-*m*CPBA

The stopped-flow technique is very useful in detecting short-lived CL. It ensures a rapid and reproducible mixing of reagents and allows full intensity versus time profiles to be measured. The stopped-flow method is also suitable for analyzing the CL reaction kinetics [11]. The CL intensity versus time profiles for the oxidation of 50 μM luminol with 10 μM *m*CPBA or 10 μM H_2O_2, catalyzed by 2.4 μM MP8 in 5.0 mM carbonate buffer at pH 12.0, are shown in Fig. 1. A much more intense CL emission with ca. 20-fold less duration was observed when using *m*CPBA as an oxidant compared to H_2O_2. The relative peak CL intensity (*m*CPBA/H_2O_2) was determined to be 12.3±0.2 (n=6).

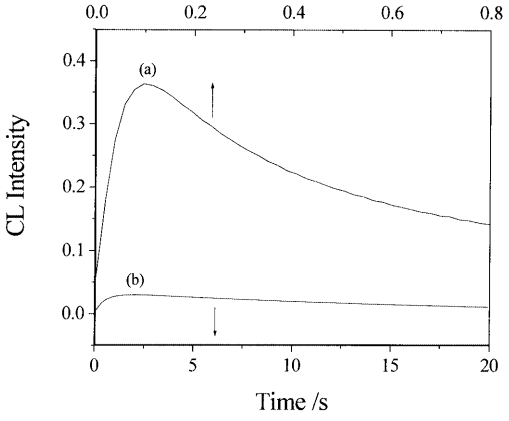

Fig. 1 CL intensity vs. time profiles for the MP8-catalyzed oxidation of luminol in 5.0 mM carbonate buffer at pH 12.0 using (**a**) *m*CPBA or (**b**) H_2O_2 as the oxidant. The concentration of MP8, oxidant, and luminol are 2.4, 10.0, and 50.0 μM, respectively. The upper time scale (also in s) was used for the CL reaction involving *m*CPBA. The PMT voltage was 500 V

Double exponential fitting of the stopped-flow traces using the built-in software of the instrument allows the estimation of the area under the curve, which is a measure of the total CL emitted. The area for trace (a) is nearly the same as that of trace (b). The results indicate that the high CL intensity involving *m*CPBA is achieved at the expense of the reduction in CL duration, suggesting that the CL enhancement is probably related to the acceleration of the CL cycle.

*m*CPBA is known to be much more efficient than H_2O_2 in transferring an oxygen atom to a heme or porphyrin iron (III) moiety to form a reactive intermediate [12]. The stopped-flow technique was utilized to measure the formation rate of the intermediate for the oxidation of MP8 with either *m*CPBA or H_2O_2. Fig. 2A shows the time-resolved difference absorption spectra for the reaction of 2.4 μM MP8 and 10.0 μM H_2O_2 in 5 mM phosphate buffer at pH 12.0. Upon addition of H_2O_2 to MP8, the appearance of three new bands at 417, 522, and 549 nm and a concomitant disappearance of the peak at 398 nm were observed. These spectral features are characteristic of the formation of the intermediate O=Fe(IV)MP8 from MP8, analogous to the formation of compound II of horseradish peroxidase or catalase [13]. Identical spectral changes were also observed when using *m*CPBA as the oxidant (data not shown), suggesting the formation of the same intermediate. The rate of formation of the intermediate can be determined by following the change in absorbance at 417 nm (A_{417}) as illustrated in Fig. 2B. The relative rate constant (*m*CPBA/H_2O_2), obtained from the single exponential fitting of the stopped-flow traces, is 19.2±0.5 (n=10). Rapid formation of the intermediate of MP8 when using *m*CPBA as the oxidant will accelerate the CL cycle, which in turn will lead to a drastic increase in CL emission in a short period of time. The discrepancy (ca. 40%) between relative rate constant and relative peak CL intensity (i.e., 19.2 versus 12.3) may result from different CL efficiencies for the subsequent reactions of luminol radicals with the two oxidants.

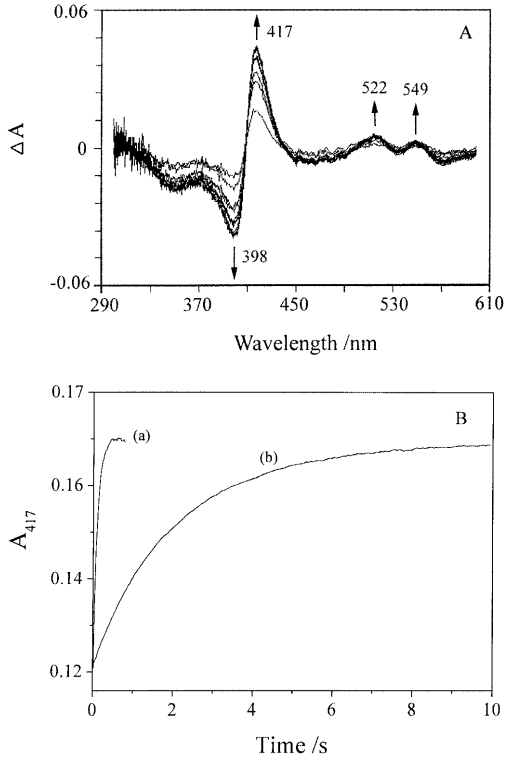

Fig. 2 **A** Stopped-flow time-resolved difference spectra for the oxidation of MP8 with H_2O_2 at pH 12.0. The time interval per trace is 0.8 s. **B** Time course of the change in absorbance at 417 nm (A_{417}) for the oxidation of MP8 with (a) *m*CPBA and (b) H_2O_2 at pH 12.0. The concentrations of the reagents are the same as those given in Fig. 1

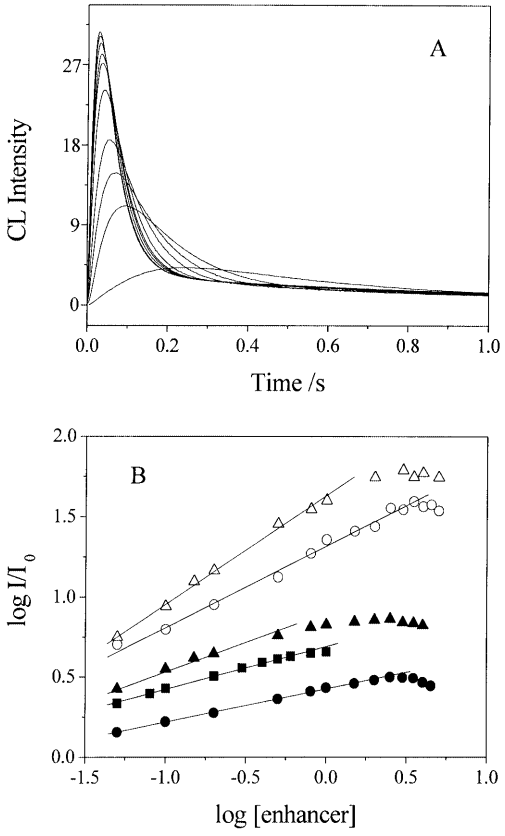

Fig. 3 **A** Stopped-flow CL intensity versus time profiles for the reactions of 2.5 μM MP8, 2.5 μM *m*CPBA, 50 μM luminol in 5 mM phosphate buffer (pH 12.0) in the presence of 0, 0.05, 0.1, 0.15, 0.2, 0.5, 0.8, 1.0, 1.5, 2.0 and 2.5 M GdnHCl (from bottom to top). The PMT voltage was 500 V. **B** The double-log plot of the relative peak CL intensity versus the concentration of NaCl (*closed circles*), Na_2CO_3 (*closed squares*), and GdnHCl (*closed triangles*) at pH 12.0 and Na_2CO_3 (*open circles*) and GdnHCl (*open triangles*) at pH 10.0

Enhancers for the CL of MP8-luminol-*m*CPBA

We have found that the addition of GdnHCl, Na_2CO_3, or NaCl dramatically enhances the CL emission of the MP8-luminol-*m*CPBA system. Fig. 3A shows the CL intensity versus time profiles for the reactions of 2.4 μM MP8, 2.4 μM *m*CPBA, and 50 μM luminol in 5 mM phosphate buffer (pH 12.0) in the presence of various concentrations of GdnHCl. As the concentration of GdnHCl increases, the CL intensity increases, whereas the CL duration decreases progressively. Fig. 3B shows the double-log plots for the relative peak CL intensity (I/I_0, where I and I_0 are the peak CL intensity in the presence and absence of enhancer, respectively) versus the concentration of enhancer. The plots are linear with slopes of 0.331, 0.278, and 0.205 for GdnHCl (0.05–1 M), Na_2CO_3 (0.005–0.8 M), and NaCl (0.05–2.5 M) at low concentration of enhancer, reach a maximum, and then drop slightly at high concentration. The relative peak CL intensities are 6.2, 4.9, and 3.7 for 2.5 M GdnHCl, 1 M Na_2CO_3, and 3 M NaCl, respectively.

The effect of the enhancer on the CL intensity of the MP8-luminol-*m*CPBA system is more pronounced at pH 10.0 than at pH 12.0 as shown in Fig. 3B. The double-log plots at pH 12.0 are linear at low concentration of enhancer with slopes of 0.66 and 0.50 for GdnHCl and NaCl, respectively, and level off at high concentration. The plot for Na_2CO_3 is very close to that for GdnCl and is not shown for the sake of clarity. The maximum CL-enhancements (CL_1) caused by the three enhancers are given in Table 1. The values of CL_1 are 59.8, 48.6, and 37.3 for GdnHCl, Na_2CO_3, and NaCl, respectively. For a given enhancer, the CL-enhancement at pH 10.0 is about an order of magnitude greater than that observed at pH 12.0. In con-

Table 1 Effect of enhancer on the values of CL_{1r}[a], CL_{1r}[b], R_{1r}[c], and R_{2r}[d]

	CL_1	CL_2	R_{1r}	R_{2r}
None	1.0	1.0	1.0	1.0
NaCl	37.3	27.0	1.24±0.04	0.38±0.01
Na_2CO_3	48.6	31.0	1.68±0.05	0.43±0.01
GdnHCl	59.8	7.4	7.06±0.25	0.12±0.01

[a]C_{1r}: peak CL intensity for MP8-*m*CPBA-luminol
[b]C_{2r}: peak CL intensity for intermediate-luminol
[c]R_{1r}: initial rate of intermediate formation for the reaction of MP8 and *m*CPBA
[d]R_{2r}: initial rate for the reaction of intermediate and luminol

trast to the progressive reduction in CL duration observed at pH 12.0, the presence of the enhancer caused no significant change in CL duration at pH 10.0 (data not shown).

pH dependence of CL intensity

The CL emission and the effect of the enhancer on CL intensity are strongly pH dependent. The pH dependence of the peak CL intensity for the CL reactions in the absence or presence of enhancers is illustrated in Fig. 4A. The plots are nearly bell-shaped. The maximum CL intensity occurs at pH 12.5 in the absence of an enhancer, 12.0 in the presence of NaCl or Na_2CO_3, and 11.5 in the presence of GdnHCl. It is noted that the peak CL intensity is enhanced to various extents for different enhancers and at different pHs. A quantitative description of the enhancement factor (defined as the ratio of the peak CL intensity in the presence of an enhancer relative to that in its absence) at various pH is demonstrated in Fig. 4B. The maximum enhancement occurs at pH 10.0 for all enhancers studied. Departure of the pH from 10.0 causes a sharp drop in the CL enhancement. Despite the high CL enhancement, the peak CL intensity at pH 10.0 in the presence of 2.5 M GdnHCl is still smaller than that observed at pH 11.5 by nearly an order of magnitude (Fig. 4A). Therefore, the most sensitive CL assays involving MP8-*m*CPBA is achieved by carrying out the reaction in the presence of 2.5 M GdnHCl at pH 11.5.

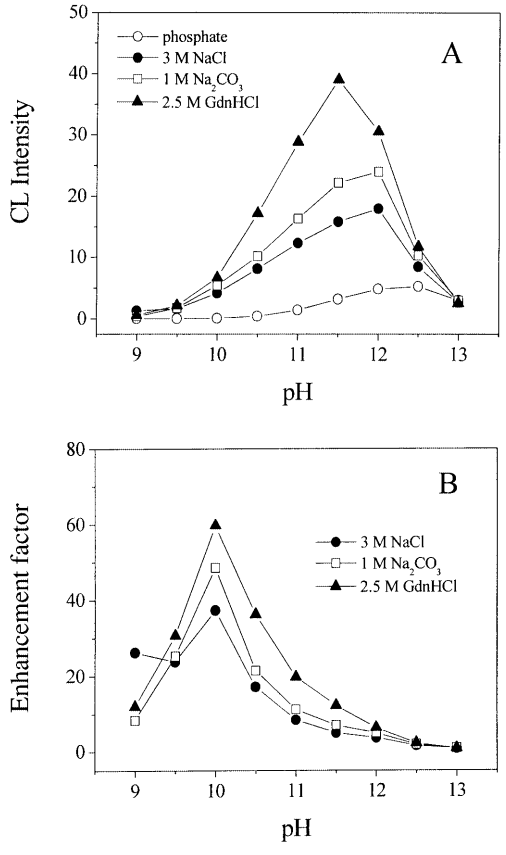

Fig. 4 **A** pH dependence of the peak CL intensities for the reactions of 2.5 μM MP8, 2.5 μM *m*CPBA, and 50 μM luminol in 5 mM phosphate buffer in the presence of phosphate (*open circles*), 3 M NaCl (*closed circles*), 1 M Na_2CO_3 (*open squares*), and 2.5 M GdnHCl (*closed triangles*). **B** pH dependence of the enhancement factor obtained by using the data in (**A**)

Effect of the concentration of *m*CPBA and luminol on the CL intensity

Since the maximum CL intensity occurs at pH 11.5–12.0 in the presence of enhancers, the dependence of CL signal on the concentrations of *m*CPBA and luminol were performed at pH 12.0. The CL intensity versus time profiles at various concentrations of *m*CPBA (data not shown) shows that the CL intensity increases rapidly as the concentration of *m*CPBA increases, whereas the CL duration remains nearly constant. When *m*CPBA is the limiting reagent, the CL intensity is proportional to the concentration of *m*CPBA as expected. The double-log plots of the peak CL intensities for the reactions in the presence and absence of 2.5 M GdnHCl at various concentrations of *m*CPBA (0.025–100 μM) are shown in Fig. 5. The plots exhibit two linear parts with slopes of 1.33 and 0.48 for the CL reaction in the presence 2.5 M GdnHCl, and 1.44 and 0.63 for the CL reaction without the enhancer. It is noted that the presence of 2.5 M GdnHCl enhances the CL emission by nearly an order of magnitude over the entire concentration range of *m*CPBA. Similar double-log plots with two linear segments (in between the two curves in Fig. 5) were also observed for the enhancer Na_2CO_3 or NaCl (data not shown).

The double-log plots of the peak CL intensities for the reactions in the presence and absence of 2.5 M GdnHCl at various concentrations of luminol (0.01–100 μM) are shown in Fig. 6. The plots are linear (at concentration of luminol below 10^{-5} M) with slopes of 1.27 and 1.18 for the CL reactions in the presence and absence of 2.5 M GdnHCl, respectively. Similar double-log plots with two linear segments (in between the two curves in Fig. 6) were also observed for the enhancer Na_2CO_3 or NaCl (data not shown).

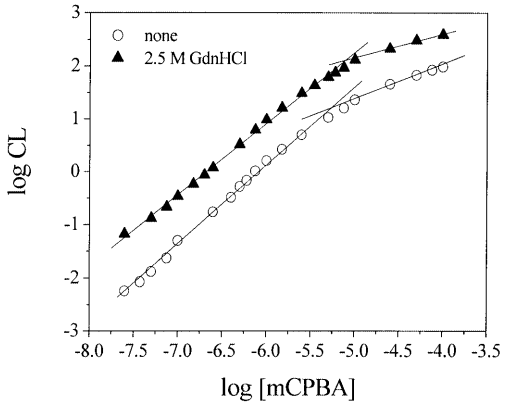

Fig. 5 The double-log plot of the peak CL intensity versus the concentration of *m*CPBA in the presence of none (*open circles*) and 2.5 M GdnHCl (*closed triangles*)

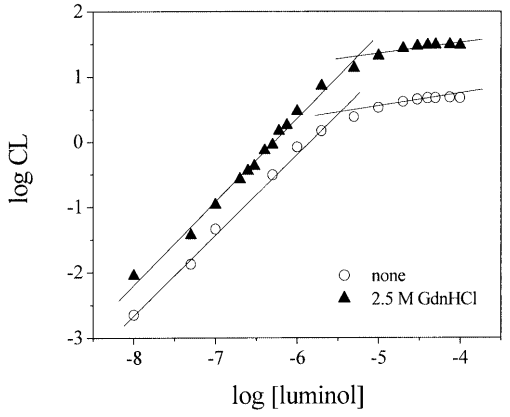

Fig. 6 The double-log plot of the peak CL intensity versus the concentration of luminol in the presence of none (*open circles*) and 2.5 M GdnHCl (*closed triangles*)

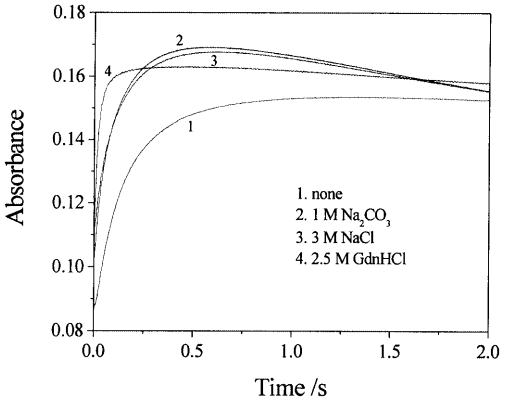

Fig. 7 Stopped-flow time courses for the changes in absorbance at 417 nm in 5 mM phosphate buffer (pH 10.0) for the oxidation of 2.5 μM MP8 by 2.5 μM *m*CPBA in the presence of none (trace 1), 1.0 M Na_2CO_3 (trace 2), 3.0 M NaCl (trace 3), and 2.5 M GdnHCl (trace 4). The PMT voltage was set at 200 V

Effect of enhancers on the rate of formation of the intermediate

Luminol is a diprotic acid (denoted as LH_2) with pK_as of 6 and ca. 13 [14]. In the pH range of 9–13 employed in this study, luminol exists mostly as LH^-. The mechanism for the CL of peroxidase-luminol-H_2O_2 is well known [15]. It generally involves the following steps:

$$MP8 + H_2O_2 \rightarrow X + OH^- \quad (1)$$

$$X + LH^- + OH^- \rightarrow MP8 + L^{-\cdot} + H_2O \quad (2)$$

$$2L^{-\cdot} + H_2O \rightarrow L + LH^- + OH^- \quad (3)$$

$$L + H_2O_2 \rightarrow 3 - AP + N_2 + \text{light} \quad (4)$$

The first step is the transfer of an oxygen atom from the oxidant to peroxidase to form a reactive intermediate (X). The second step is the abstraction of a hydrogen atom from luminol (LH^-) by the intermediate to produce luminol radical-anion ($L^{-\cdot}$). Step 3 is the disproportionation of two luminol radicals to yield diazaquinone (L) and luminol (LH^-). Step 4 is the reaction of diazaquinone with H_2O_2 to give 3-aminophthalate (3-AP), accompanied by an emission of light. Since *m*CPBA and H_2O_2 form the same intermediate when reacted with MP8, it is reasonable that a similar CL mechanism also applies to MP8-luminol-*m*CPBA by replacing H_2O_2 with *m*CPBA. Measuring the rates of formation and disappearance of the intermediate (i.e., steps 1 and 2) in the presence and absence of enhancers is helpful for an understanding of the CL enhancement. Since the maximum CL enhancement occurs at pH 10.0, the kinetic measurements were carried at this pH. Fig. 7 shows the time course for the change in absorbance at 417 nm for the reaction of MP8 and *m*CPBA in 5.0 mM phosphate buffer (pH 10.0) in the absence or presence of enhancers. Single exponential fitting of the curves allows the calculation of the initial rate of step 1 (R_1). The relative values of R_1 obtained in the presence and absence of an enhancer (denoted by R_{1r}) are given in Table 1. The values of R_{1r} are 7.06, 1.68, and 1.24 for GdnHCl, Na_2CO_3, and NaCl, respectively. The presence of enhancer accelerates the formation of intermediate.

The sequential mixing stopped-flow equipment allows the rate for the reaction of intermediate and luminol (i.e., step 2) to be measured. Single exponential fitting of the stopped-flow traces (data not shown) allows the calculation of the initial rate for step 2 (R_2). The relative values of R_2 obtained in the presence and absence of an enhancer (denoted by R_{2r}) are also given in Table 1. The values of R_{2r} are 0.12, 0.43, and 0.38 for GdnHCl, Na_2CO_3, and NaCl, respectively. The presence of enhancers actually decreases the rate of step 2.

The combined effect of the enhancer ($R_{1r} \times R_{2r}$) on the first two steps of the CL mechanism leads to a slight deceleration of the CL cycle, which will tend to diminish the CL emission. Consequently, the enhancement in CL must be due to the effect of the enhancer on the last two steps. The relative peak CL intensities (CL_2) obtained from the CL intensity versus time profiles (data not shown) for the reactions of luminol and the intermediate in the presence and absence of enhancers are listed in Table 1. The values of CL_2 are 7.4, 31.0, and 27.0 for 2.5 M GdnHCl, 1 M Na_2CO_3, and 3 M NaCl, respectively. Evidently, the presence of the enhancers causes a dramatic increase in the CL emission, despite the slow-down of step 2. Thus, the presence of enhancers must have a profound effect on the last two steps of the CL mechanism. It is noted that step 3 involves the reaction of an ionic species of the same charge and will be accelerated significantly by the increasing ionic strength. Moreover, the enhancer may also have a prominent effect on the CL efficiency of step 4. The CL emission of the MP8-luminol-*m*CPBA system is much less efficient at pH 10.0 than at pH 12.0 (Fig. 4A) in the absence of the enhancer, probably because of the poor efficiencies of the last two steps. Consequently, the improvement of the efficiencies of these two steps by the presence of the enhancers can dramatically enhance the CL emission without significantly affecting the CL duration.

Similar experiments for measuring the rates of formation of the intermediate and the subsequent reaction of the

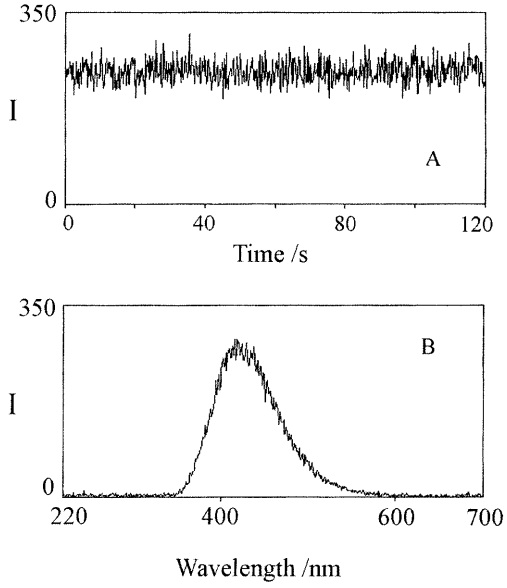

Fig. 8 **A** The time scan of the CL intensity and **B** the CL emission spectrum for the reaction of 2.5 μM MP8, 2.5 μM *m*CPBA, 50 μM luminol, 2.5 M GdnHCl in 5 mM phosphate buffer at pH 12.0. The PMT voltage was set at 700 V, the bandpass 10 nm, the emission wavelength (for (**A**)) 425 nm, and the scan rate (for (**B**)) 240 nm min^{-1}

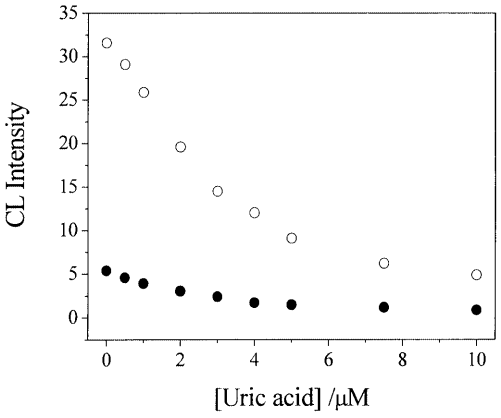

Fig. 9 Plot of the peak CL intensity versus the concentration of uric acid for the reactions of 2.5 μM MP8, 2.5 μM *m*CPBA, and 50 μM luminol in 5 mM phosphate buffer (pH 12.0) and in the presence (*open circles*) or absence (*closed circles*) of 2.5 M GdnHCl

intermediate with luminol were also carried out at pH 12.0 (data not shown). The relative rates of step 1 (R_{1r}) are 8.76±0.16, 3.36±0.06, and 2.97±0.07, and those of step 2 (R_{2r}) are 0.66±0.01, 3.37±0.06, and 2.95±0.06 for 2.5 M GdnHCl, 1 M Na_2CO_3, and 3 M NaCl, respectively. The combined effect of the enhancer on the rates of these two steps is very close to the observed CL enhancement for GdnHCl (5.8 versus 6.2) and is about twice the observed CL enhancement for Na_2CO_3 (11.3 versus 4.9) and NaCl (8.8 versus 3.7). The results indicate that the contribution from the first two steps of the CL mechanism in accelerating the CL cycle plays a major role in the CL enhancement. Moreover, since the CL emission is very efficient at pH 12.0 even in the absence of the enhancer, it is likely that a maximum total CL emission has already been achieved for the given reaction conditions. For a constant total CL emission, a fast CL cycle will thus result in a reduction in CL duration (Fig. 3A).

Measurement of the CL emission spectrum is important in understanding the CL mechanism. The CL emission spectrum was measured using a continuous flow manifold as described previously [10]. By adjusting the flow rate, a steady-state CL signal was achieved for the CL reaction of 2.5 μM MP8, 2.5 μM *m*CPBA, 50 μM luminol, 2.5 M GdnHCl at pH 12.0 as illustrated in Fig. 8A. With the establishment of the steady-state signal, the CL emission spectrum can then be determined using a fluorimeter as illustrated in Fig. 8B. Similar CL emission spectra were also obtained when using other enhancers (Na_2CO_3 or NaCl). The emission spectrum exhibits a maximum at 425 nm, which is the characteristic emission for the excited 3-aminophthalate [14]. No other emission bands were observed. Therefore, the product of luminol (i.e., 3-aminophthalate) is responsible for the emission in this CL system.

Detection of uric acid

The CL system can be applied to detect any one of its reaction components, including CL substrate, oxidant, and catalyst. Moreover, any reagents that will enhance or inhibit the CL emission can also be determined. As an illustration of the application of this enhanced CL system, the detection of uric acid (a natural antioxidant in the plasma) was demonstrated. Fig. 9 shows the plots of the peak CL intensities at various concentrations of uric acid (0–10 μM) for the oxidation of 50 μM luminol with 2.5 μM *m*CPBA catalyzed by 2.5 μM MP8 at pH 12.0 and in the presence or absence of 2.5 M GdnHCl. The addition of uric acid significantly diminishes the CL emission, allowing the determination its concentration. The double-log plots are linear for both curves in Fig. 9 (data not shown). Upon addition of uric acid, not only the CL signal itself but also its change are much greater when GdnHCl is present as compared to its absence. The result demonstrates the feasibility of employing this CL system as a sensitive means for the determination of uric acid, especially when GdnHCl is present.

Conclusion

The use of *m*CPBA in stead of H_2O_2 can increase the CL emission of MP8-luminol by an order of magnitude and the presence of the enhancer (GdnHCl, Na_2CO_3, or NaCl) can further enhance the CL emission significantly. The acceleration of the formation of the reactive intermediate of MP8 plays an important role in the CL enhancement. Thus, the CL system of MP8-luminol-*m*CPBA is far more sensitive than that of MP8-luminol-H_2O_2, especially in the

presence of an appropriate enhancer. This CL system can be employed to determine any one of its reaction components, including those that can enhance or inhibit the CL emission.

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