Enhancement in Chemiluminescence by Carbonate for Cobalt(II)-catalyzed Oxidation of Luminol with Hydrogen Peroxide

Hui-Chun Yeh (葉慧君), Wen-Tong Hsu (許文東) and Wann-Yin Lin* (林萬寅)
Department of Chemistry, National Taiwan University, Taipei 106, Taiwan, R.O.C.

Chemiluminescence (CL) from the cobalt(II)-catalyzed oxidation of luminol with hydrogen peroxide was dramatically enhanced by the presence of carbonate. The CL signal increases by several orders of magnitude over a wide range of concentrations of Co(II), luminol, or hydrogen peroxide. A limit of detection of 10^{-12} M for Co(II) and luminol and 10^{-8} M for hydrogen peroxide can be achieved. The CL emission spectrum exhibits a maximum at 425 nm, indicating that the excited 3-aminophthalate is the emitting species. ESR spin-trapping experiments revealed a large increase in the production of hydroxyl and carbonate radicals by the presence of carbonate, which is responsible for the enormous CL enhancement. Uric acid, ascorbic acid, acetaminophen, and *p*-hydroxyphenyl acetic acid are capable of scavenging the radicals, thereby inhibiting the CL emission. The inhibition of CL intensity can be used to determine these substances at the sub-micromolar level.

Keywords: Chemiluminescence; Stopped-flow; Co(II)-Luminol-H_2O_2; Carbonate.

INTRODUCTION

Chemiluminescence (CL) based on luminol and hydrogen peroxide has been employed for the determination of a variety of substances, such as metal ions, anions, H_2O_2, glucose, cholesterol, etc.[1-3] The advantages of CL methods include superb sensitivity, wide dynamic range, low background noise, simple instrumentation, and easy automation. CL from the oxidation of luminol with various oxidants is one of the best known and most useful systems. It is usually carried out in an alkaline solution in the presence of a suitable catalyst, such as metal ions, metal complexes, or peroxidases.[4-8] A number of substances have been developed to reduce the detection limit of the CL assays involving luminol. For example, phenolic derivatives, phenylboronic acids, and chemical indicators are effective enhancers for HRP-luminol-H_2O_2,[9-11] sodium chloride for Mn(II)-luminol,[12] carbon dioxide for Co(II)-luminol,[13] halides for metal ion-luminol-H_2O_2,[14] and carbonate and bromide for Cr(III)-luminol-H_2O_2.[15] Scientists are continuing to search for reagents that will enhance the CL intensity.

In this paper, we report the enormous enhancement by carbonate on the CL emission for cobalt(II)-catalyzed oxidation of luminol. We have systematically investigated the effect of the concentrations of cobalt(II), luminol, and hydrogen peroxide on the CL intensity. We also discussed the possible origin of the CL enhancement from the results of CL emission spectrum and ESR spin-trapping of hydroxyl radicals. Finally, we employed this CL system to determine the antioxidants at the sub-micromolar level.

EXPERIMENTAL SECTION

Materials

Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) and sodium dihydrogen phosphate were purchased from Sigma (St. Louis, MO, USA). Sodium carbonate and D-mannitol were obtained from Acros Organics Company (Geel, Belgium). Cobalt(II) chloride hexahydrate ($CoCl_2 \cdot 6H_2O$), iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), manganese(II) chloride dihydrate ($MnCl_2 \cdot 2H_2O$), copper(II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$), nickel(II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$), magnesium(II) chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), zinc(II) chloride ($ZnCl_2$) were purchased from Merck (Darmstadt, Germany). All reagents were of the highest grade of purity. Ultra high purity of 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,

Dedicated to Professor Ching-Erh Lin on the Occasion of his 66th Birthday and his Retirement from National Taiwan University

* Corresponding author. Tel: +886-2-23638001; Fax: +886-2-23696359; E-mail: wylin@ntu.edu.tw

Seelze, Germany) was prepared by volumetric dilution of 30% (w/w) H_2O_2 by deionized water. 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 $\varepsilon_{240} = 39.4\ M^{-1}\ cm^{-1}$.[16] The stock solutions of luminol and other reagents were prepared by dissolving a precisely weighed solid in 1 M carbonate and adjusted to pH 10.0. The stock solutions (1 mM each) of metal ions were prepared in deionized water. In order to keep the Fe(II) in solution, it was made 1 M in HCl.

Instruments

All the CL measurements were performed on a stopped-flow spectrofluorimeter (Hitech SF-61DX2, Hitech Scientific, Salisbury, UK) as described previously.[17] The PMT voltage was set at 700 V or 900 V, depending on the magnitude of the CL signal. The cobalt(II) solution was stored in one syringe and the luminol-H_2O_2 mixture in the other. All experiments were carried out at 25 °C. The CL emission spectra were measured using a two-channel continuous-flow manifold as described.[17] Co(II) solution flows in one channel and luminal/H_2O_2 mixture in the other. The CL emission spectrum was recorded with a fluorescence spectrophotometer (F-2000, Hitachi, Japan) via a flow cell. Electron spin resonance (ESR) spectra were measured with a Bruker ECS 106 spectrometer (Bruker Instruments, Billerica, MA, USA) operated at 9.8 GHz with a modulation frequency of 50 kHz at room temperature. The spectrometer gain was set at 1.0×10^4 with a modulation amplitude of 1.0 G and a microwave power of 50 mW. The sweep time was 83.9 s and was centered at 3440 G with a sweep width of 100 G.

RESULTS AND DISCUSSION

Enhancement in CL emission by bicarbonate

Stopped-flow is a very powerful technique for detecting the short-lived CL. It ensures a rapid and reproducible mixing of reagents and allows the recording of full intensity versus time profiles. Fig. 1 shows the stopped-flow CL intensity versus time profiles for the reactions of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol at pH 10.0 in the presence of various concentrations of carbonate. At pH 10.0, bicarbonate and carbonate exist at a ratio of 2:1 (pK_2 of carbonic acid is 10.3). In the absence of carbonate, essentially no CL signal was detected. Upon addition of carbonate, a dramatic enhancement in CL emission was observed as shown in Fig. 2a. Most CL assays involving luminol and H_2O_2 were carried out at relatively high concentrations of both reagents (mM or higher) to achieve a measurable CL signal. However, when the reagent concentrations are relatively low (5 μM luminol, 10 μM H_2O_2 in this study), the CL emission can only be observed in the presence of carbonate. The double-log plot for the peak CL intensity versus the concentration of carbonate (5 mM ~ 1 M) is shown in Fig. 1b. The plot is linear and covers two orders of magnitude in concentration and three orders of magnitude in CL intensity. The pH-dependence of CL intensity is bell-shaped with a maximum at ~ pH 10.0 (data not shown). Therefore all the CL experiments were performed at pH 10.0 throughout this study.

Effects of various reagent concentrations on the CL emission

The CL emission is strongly dependent on the concen-

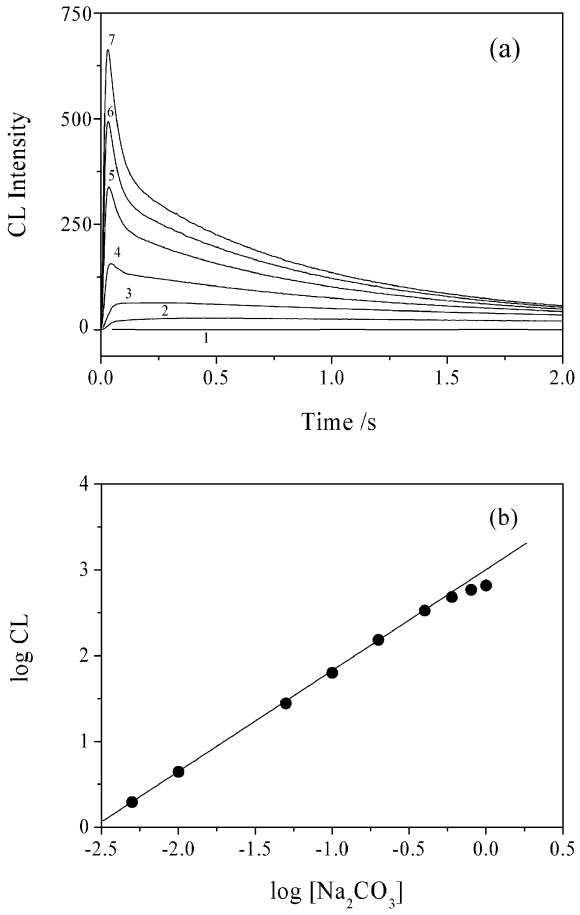

Fig. 1. (a) Stopped-flow CL intensity vs. time profiles for the reactions of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol (pH 10.0) in the presence of 0, 0.05, 0.1, 0.2, 0.4, 0.6, and 1.0 M carbonate (traces 1 to 7). (b) The double-log plot of peak CL intensity vs. the concentration of carbonate.

trations of Co(II), luminol and H_2O_2. Typical CL intensity versus time profiles for the reactions of 10 μM H_2O_2, 5 μM luminol, 1 M carbonate (pH 10.0) at various concentrations of Co(II) are illustrated in Fig. 2a. Essentially no CL emission was observed in the absence of Co(II) (trace 1 in Fig. 2a). The CL intensity increases rapidly as the concentration of Co(II) increases. The double-log plot for the peak CL intensity versus the concentration of Co(II) shown in Fig. 2b exhibits two linear portions with slopes of 0.086 ($10^{-12} \sim 10^{-8}$ M) and 1.176 ($10^{-8} \sim 10^{-5}$ M). The CL intensity covers nearly four orders of magnitude over the entire concentration range of Co(II) studied. Thus the CL system allows the determination of Co(II) at $10^{-8} \sim 10^{-5}$ M with high sensitivity and $10^{-12} \sim 10^{-8}$ M with reasonable sensitivity owing to the good precision of the data obtained from stopped-flow experiments.

The dependence of CL signal on the concentration of luminol is demonstrated in Fig. 3a for the reaction of 10 μM Co(II), 10 μM H_2O_2, and 1 M carbonate (pH 10.0). The CL signal and the total amount of CL emission (i.e., the area under the CL profile) increase rapidly as the concentration of luminol increases. It is noted that the shape of the CL profile depends on the concentration of luminol. At 0.1 μM luminol, a normal rise and decay curve corresponding to the formation and destruction of an emitting species was observed (trace 2 in Fig. 3a). At concentrations above 0.5 μM (traces 3-5), a biphasic decay of CL emission was observed after an initial rise. The overall CL profile can be considered as a combination of two rise-and-decay curves, indicating the existence of two emitting processes. The fast one remains fairly constant from trace 3 to trace 5, while the second one increases steadily, as the concentration of luminol increases. The dou-

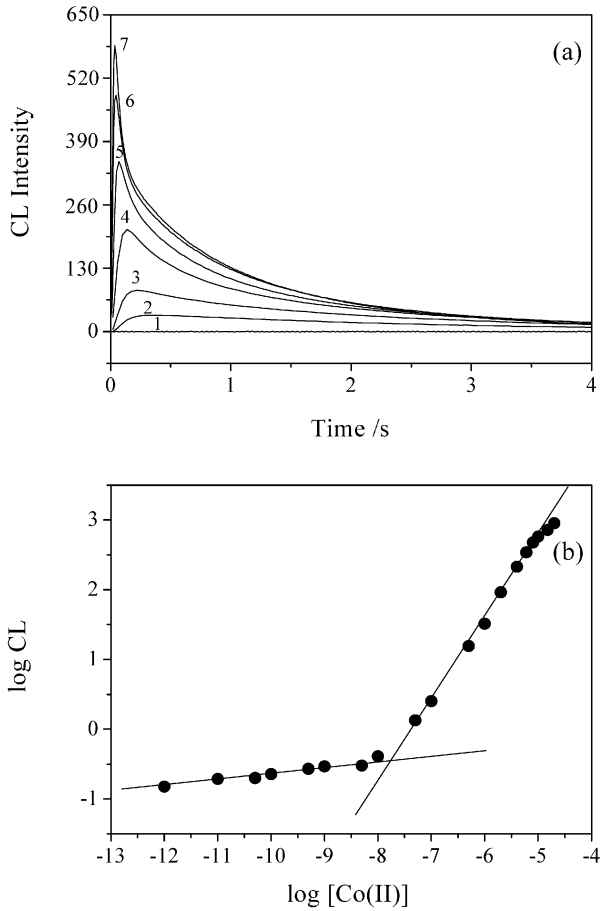

Fig. 2. (a) Stopped-flow CL intensity vs. time profiles for the reactions of 10 μM H_2O_2, 5 μM luminol, 1 M carbonate (pH 10.0) in the presence of 0, 1, 2, 4, 6, 8, and 10 μM Co(II) (traces 1 to 7). (b) The double-log plot of peak CL intensity vs. the concentration of Co(II).

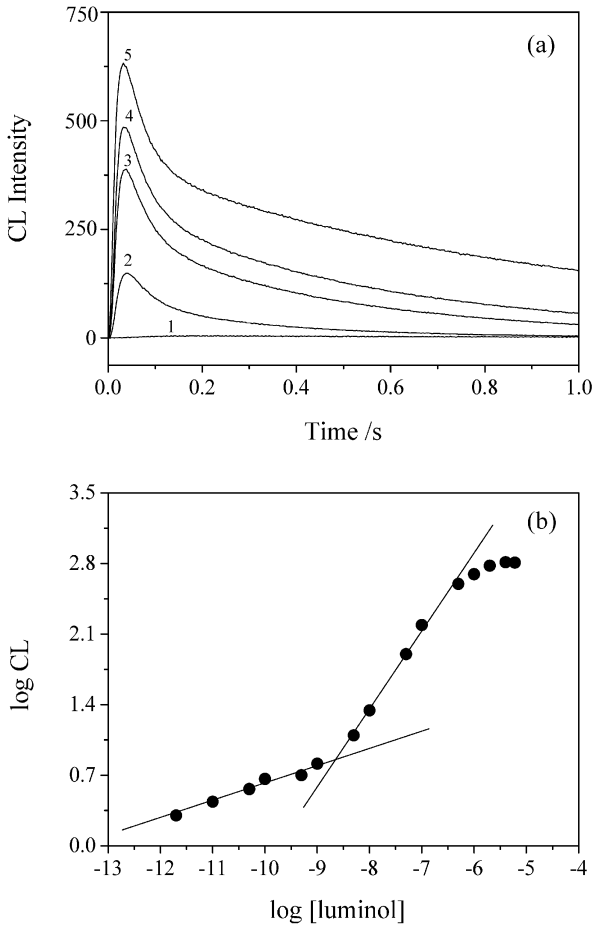

Fig. 3. (a) Stopped-flow CL intensity vs. time profiles for the reactions of 10 μM Co(II), 10 μM H_2O_2, 1 M carbonate (pH 10.0) in the presence of 0, 0.1, 0.5, 1.0, and 4.0 μM luminol (traces 1 to 5). (b) The double-log plot of peak CL intensity vs. the concentration of luminol.

ble-log plot for the peak CL intensity versus the concentration of luminol (Fig. 3b) also shows two linear portions with slopes of 0.181 (10^{-12} ~ 10^{-9} M) and 0.765 (10^{-9} ~ 10^{-6} M), respectively.

Fig. 4a shows the CL intensity versus time profiles for the reactions of 10 μM Co(II), 5 μM luminol, and 1 M carbonate (pH 10.0) at various concentrations of H_2O_2. The presence of an increasing amount of H_2O_2 causes a dramatic increase both in CL signal and in total amount of CL emission. The double-log plot for the peak CL intensity versus the concentration of H_2O_2 (Fig. 4b) is linear over the concentration range of 10^{-8} ~ 5×10^{-4} M. The CL intensity covers six orders of magnitude over the entire concentration range of H_2O_2 studied. The results from the dependence of CL signal on the concentrations of Co(II), luminol, and H_2O_2 indicate that an intense CL emission can only be obtained when all three reagents are present.

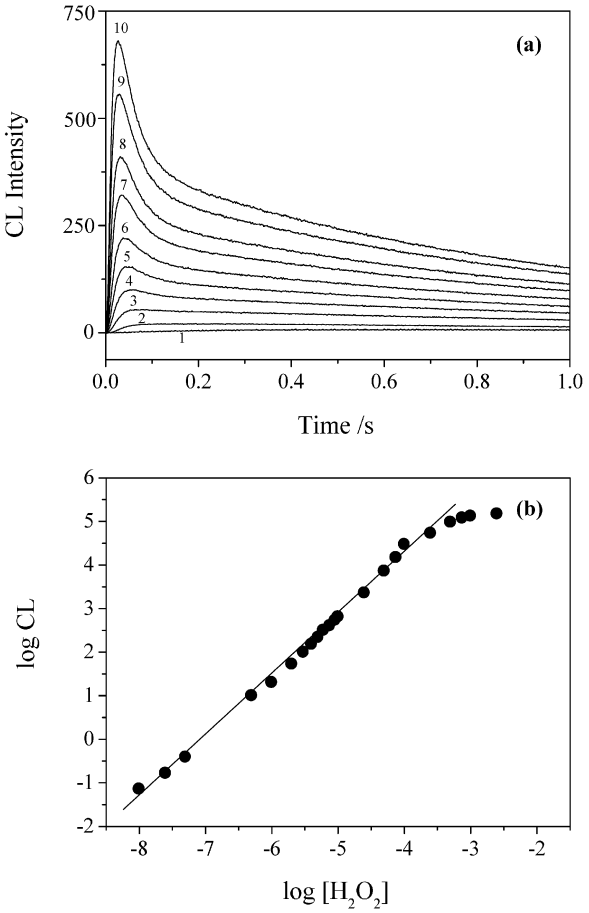

Fig. 4. (a) Stopped-flow CL intensity vs. time profiles for the reactions of 10 μM Co(II), 5 μM luminol, 1 M carbonate (pH 10) in the presence of 0, 0.98, 1.96, 2.94, 3.92, 4.9, 5.88, 7.35, 8.82, and 9.8 μM H_2O_2 (from trace 1 to 10). (b) The double-log plot of peak CL intensity vs. the concentration of H_2O_2.

Detection of picomolar Co(II) and luminol

With the presence of 1 M carbonate in the CL system, very low concentrations of Co(II), luminol, and H_2O_2 can be detected as demonstrated in Fig. 5. No CL signal was observed in the absence of Co(II) (trace I in Fig. 5a), indicating the requirement of Co(II) as the catalyst for this CL system. The presence of 1.0×10^{-12} M Co(II) in a solution containing 5 μM luminol, 10 μM H_2O_2, and 1 M carbonate (pH 10.0) results in a pronounced CL emission (trace II), allowing the detection of Co(II) at the picomolar level. Detection limits of 1.0-20 ng/L (17-340 pM) for Co(II) were reported with CL methods involving H_2O_2 and various CL reagents, including luminol, lucigenin, gallic acid, and sulfosalicylic acid.[18] Moreover, Liu, et al. reported a sub-femtomolar detection of Co(II) using capillary electrophoresis with CL detection involving luminol (1 mM) and H_2O_2 (20 mM).[19] They used a field-amplified injection (i.e., stacking of Co(II)) to enhance the CL signal by six orders of magnitude, thereby achieving a detection limit of 1.3×10^{-16} M (130 pM without stacking). The detection limit reported in this study for Co(II) is superior or at least comparable to most batch-type or flow-injection CL detections and is achieved at much lower concentrations of reagents.

Zhang, et al. have reported an intense CL signal for a mixture of Co(II), H_2O_2 (0.01 M), and $NaHCO_3$ (0.4 M) solution.[20] They ascribed the CL emission to the decomposition of a bicarbonate dimer (400-460 nm) and an oxygen dimer (490-555 nm). The weak CL signal shown in trace I of Fig. 5b for 10 μM Co(II), 10 μM H_2O_2, and 1 M carbonate (pH 10.0) is believed to arise from the same origin as observed by Zhang, et al. Addition of 1.0×10^{-12} M luminol significantly enhances the CL signal, which is accompanied by a reduction in CL duration (trace II in Fig. 5b); therefore, a detection limit of picomolar for luminol can be achieved by this CL system. In fact, the presence of 1 μM luminol can enhance the CL signal by nearly three orders of magnitude compared to the luminol-free system (Fig. 3b).

The detection of a low level of hydrogen peroxide is limited by the presence of a nanomolar of H_2O_2 from natural causes in aqueous solution. Deionized water usually contains ~ 30 nM of H_2O_2, which gradually decreases to ~ 1 nM after dark storage.[21] Spiking the solution containing 10 μM Co(II), 5 μM luminol, and 1 M carbonate (pH 10.0) with 2.4×10^{-8} M H_2O_2 results in a discernable CL emission (trace II in Fig. 5c) above the blank signal (trace I). It is difficult to determine

H_2O_2 at a concentration below 10 nM because of the severe interference from the background level of H_2O_2.

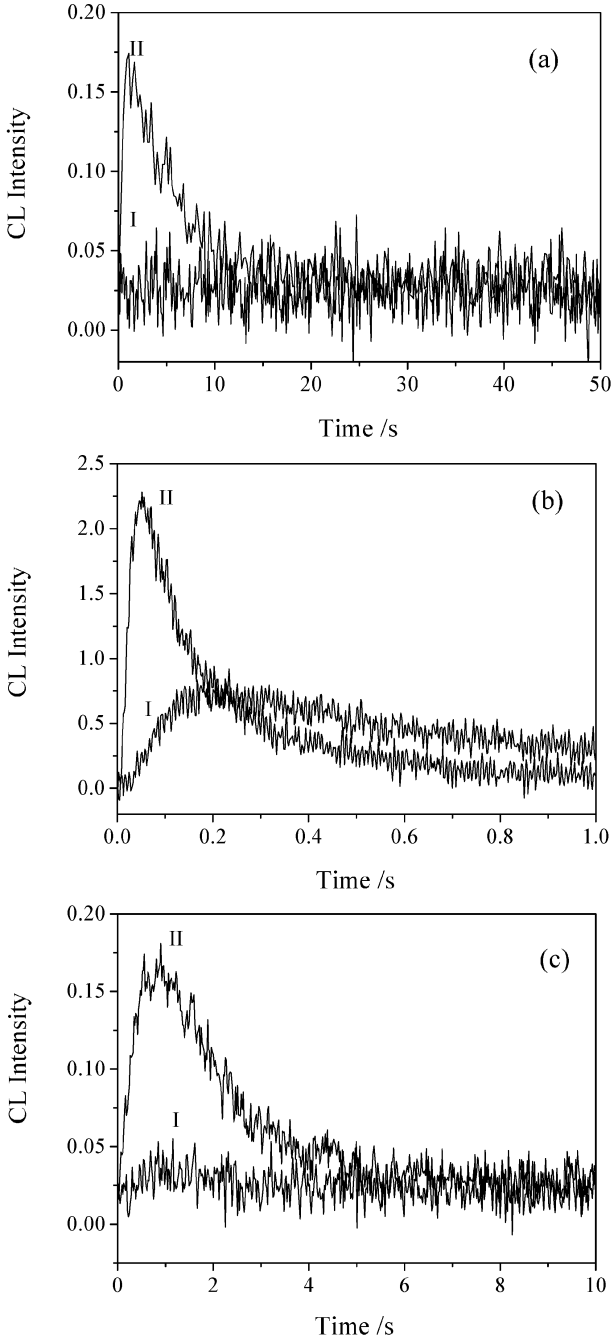

Fig. 5. Stopped-flow CL intensity vs. time profiles for the CL reactions in 1 M carbonate (pH 10.0) in the absence (trace I) and presence (trace II) of (a) 1.0×10^{-12} M Co(II), (b) 1.0×10^{-12} M luminol, and (c) 2.4×10^{-8} M H_2O_2. In each case, the two remaining reagents were chosen from 10 μM Co(II), 10 μM H_2O_2, and 5 μM luminol.

Interference tests

Besides Co(II), a number of metal ions can also induce CL emission from luminol and H_2O_2.[1,18] We have measured the CL emission from 5 μM luminol, 10 μM H_2O_2, and 1 M carbonate (pH 10.0) in the presence of 10 μM of various metal ions. No precipitation was visible for the sample solutions of these metal ions. The results indicated that none of the tested metal ions, including Fe(II), Fe(III), Ni(II), Zn(II), and Mg(II), exhibited relative CL intensity greater than 0.05% compared to that of Co(II). No CL signal was observed for Mn(II) and Cu(II). Thus this CL method is very selective for the detection of Co(II) using relatively low concentrations of luminol and H_2O_2.

We have also tested the effect of various substances on the CL emission of 10 μM Co(II), 5 μM luminol, 10 μM H_2O_2, and 1 M carbonate (pH 10.0). The results indicated that essentially no change in CL intensity (< 0.1%) was observed by the presence of 1 mM of citrate, iodide, nitrite, oxalate, phosphate, fructose, galactose, glucose, malonate, mannose, salicylate, sucrose, or sulfate. On the other hand, Tyr, Cys, Trp, His, and sulfite dramatically inhibited the CL emission, which can be used to determine these substances. Other amino acids showed no significant effect on the CL emission.

CL emission spectrum

Knowledge of the CL emission spectrum is very important for the identification of emitting species. The CL emission can be measured using a continuous-flow manifold in conjunction with a fluorescence spectrometer as described previously.[17] By adjusting the flow rate, a steady-state CL signal was obtained for the reaction of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol, and 1 M carbonate at pH 10.0 as demonstrated in Fig. 6a. With the establishment of the steady-state signal, the CL emission spectrum can then be determined by the fluorometer (Fig. 6b). The emission spectrum exhibits a maximum at 425 nm, which is the characteristic emission for the excited 3-aminophthalate.[22] No other emission bands were observed. Therefore the product of luminol (i.e., 3-aminophthalate) is responsible for the emission in this CL system.

Spin trapping of hydroxyl radical for the CL reactions

The hydroxyl radical plays an important role in the CL emission from the oxidation of luminol with H_2O_2 catalyzed by metal ions. The ESR spin trapping technique is widely used as a powerful tool for detecting radicals and DMPO is an efficient spin trapping agent for hydroxyl radicals.[23,24] The ESR spectra for 110 μM Co(II), 110 μM H_2O_2, 50 mM DMPO (pH 10.0) at various concentrations of carbonate are illus-

trated in Fig. 7. The 1:2:2:1 quartet with hyperfine splitting constants of a^N = 14.9 G and a^H = 14.9 G is the characteristic ESR signal of the DMPO-$OH^•$ adduct. The ESR signal increases nearly five fold as the concentration of carbonate increases from 0 to 1 M. In the ESR experiments, DMPO must compete with other substances for hydroxyl radicals. Carbonate and bicarbonate are known to react effectively with $OH^•$ to form carbonate radicals $CO_3^{•-}$ (eq. (1) and eq. (2)) with second-order rate constants of 3.65×10^8 and 3.6×10^7 $M^{-1}\cdot s^{-1}$, respectively.[25]

$$CO_3^{2-} + OH^• \rightarrow CO_3^{•-} + OH^- \quad (1)$$

$$HCO_3^- + OH^• \rightarrow CO_3^{•-} + H_2O \quad (2)$$

With such a high concentration of carbonate/bicarbonate and large rate constants, a large proportion of $OH^•$ will react with the carbonate species. Therefore, the hydroxyl radicals actually generated in the CL system containing 1 M carbonate will greatly exceed the amount determined from the spin trapping experiments.

Both hydroxyl and carbonate radicals are capable of reacting with luminol (represented as LH^-; luminol LH_2 is a diprotic acid with pK_a's of 6 and 13) to form luminol radicals ($L^{•-}$). Hydroxyl radicals are known to attack luminol at non-specific sties, giving rise to a high proportion of CL-silent products ($[L^{•-}]'$) according to eq. (3).

$$OH^• + LH^- \rightarrow L^{•-} + [L^{•-}]' + H_2O \quad (3)$$

Carbonate radicals, on the other hand, react very efficiently and specifically with luminol to form luminol radicals with a rate constant of 9×10^8 $M^{-1}\cdot s^{-1}$.[25]

$$CO_3^{•-} + LH^- \rightarrow CO_3^{2-} + L^{•-} + H^+ \quad (4)$$

Subsequent reactions of luminol radicals yield diazaquinone (L) and the final product 3-aminophthalte (3-AP) and CL emission (eq. (5) and eq. (6)).[26]

$$2\,L^{•-} + H_2O \rightarrow L + LH^- + OH^- \quad (5)$$

$$L + H_2O_2 \rightarrow 3\text{-AP} + N_2 + \text{light} \quad (6)$$

Thus a large increase in the production of hydroxyl and carbonate radicals by the presence of carbonate is consistent with the enormous CL enhancement.

Reaction of Fe(II) and H_2O_2 also produces hydroxyl

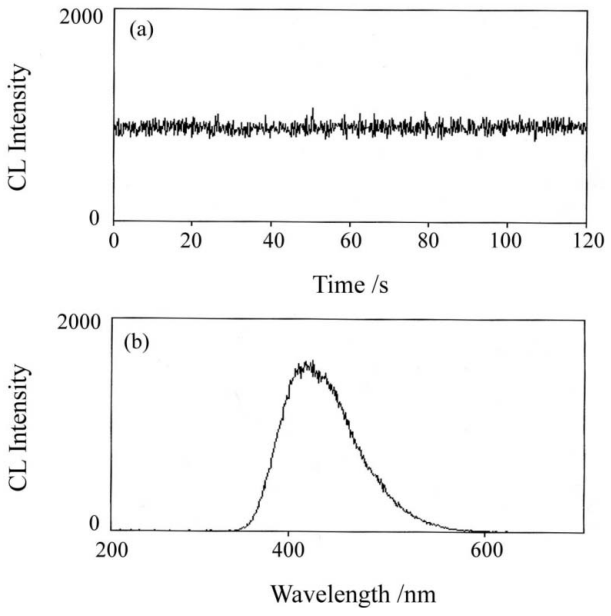

Fig. 6. (a) The time scan of the CL intensity and (b) the CL emission spectrum for the reaction of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol, 1 M carbonate at pH 10.0. The PMT voltage was set at 700 V, the slit width 10 nm, the emission wavelength 425 nm (for (a)), and the scan rate 240 nm/min (for (b)).

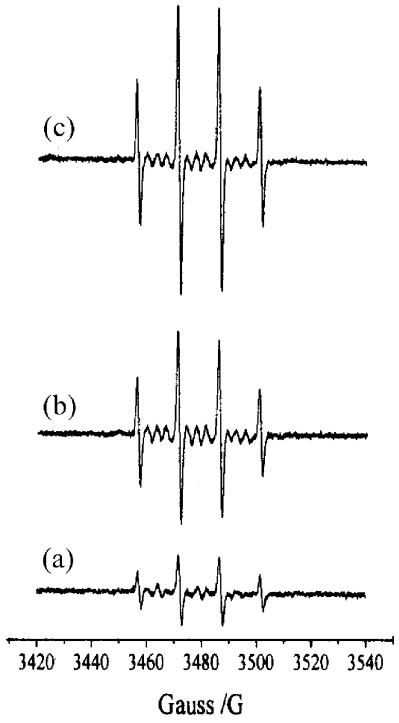

Fig. 7. ESR spectra of the DMPO-$OH^•$ adduct produced by the reaction of 110 μM Co(II), 110 μM H_2O_2, 50 mM DMPO (pH 10.0) in the presence of (a) 0 (b) 0.1 M (b) 1.0 M carbonate.

radicals efficiently and is known as the Fenton's reaction. An intense ESR signal for the DMPO-$OH^{\bullet}$ adduct was observed when 110 μM Fe(II), 110 μM H_2O_2, and 50 mM DMPO were mixed at pH 10.0. However, the ESR signal dropped nearly an order of magnitude upon addition of 1 M carbonate (data not shown). Formation of ion-pairs between Fe(II) and carbonate may inhibit the production of hydroxyl radicals. The dramatic decrease in the production of hydroxyl radicals upon addition of carbonate is consistent with its extremely weak CL emission. Metal ions other than Co(II) are unable to induce any appreciable CL emission from luminol in the presence of carbonate probably because of a reason similar to that described for Fe(II).

As mentioned earlier, the overall CL profile consists of two emitting processes and both processes occur only in the presence of luminol (Fig. 3a). The fast emitting process is very short-lived (within 1 s) and saturates at ~ 0.5 mM luminol, whereas the second process is relatively long-lived and saturates at ~ 5 mM luminol. These two processes may originate from the reaction of luminol with different radicals (e.g., $OH^{\bullet}$, $CO_3^{\bullet-}$), because the only observed emitting species is the product of luminol (Fig. 6b). However, the CL emission spectrum of the first process may be too fast to be detected. Thus the presence of other radicals or emitting species responsible for the fast process cannot be excluded.

Applications

The CL system can be employed to determine any reagent that can enhance or inhibit its emission. Any substance that scavenges the radicals will tend to inhibit the CL emission. Fig. 8a shows the inhibition of the CL emission from 10 μM Co(II), 5 μM luminol, 10 μM H_2O_2, and 1 M carbonate (pH 10.0) at various concentrations of uric acid (0.01~5 μM). At low concentrations of added uric acid (< 0.25 μM), only the fast emitting process decreases its CL intensity appreciably. The second emitting process is inhibited significantly at relatively high concentrations of uric acid (0.5~5 μM) and is probably accompanied by a delay of emission. The two-stage inhibition is seen more clearly in the double-log plots of CL intensity versus the concentration of uric acid as shown in Fig. 8b. The plot contains two linear segments with slopes of -0.183 ($1 \times 10^{-8} \sim 2.5 \times 10^{-7}$ M) and -1.498 ($5 \times 10^{-7} \sim 5 \times 10^{-6}$ M), respectively. Ascorbic acid, acetaminophen, and *p*-hydroxyphenyl acetic acid also exhibit a similar two-stage inhibition but differ in inhibition abilities as demonstrated in Fig. 9. All inhibition plots consist of two linear portions (Table 1), which can be used to determine these substances.

CONCLUSION

Intense CL emission was observed when carbonate was added to the solution containing Co(II), luminol, and hydrogen peroxide. The dramatic enhancement in CL intensity may result from a large increase in the production of hydroxyl and carbonate radicals by the presence of carbonate. Substances such as uric acid, ascorbic acid, acetaminophen, and *p*-hydroxyphenyl acetic acid are capable of scavenging the radicals, thereby inhibiting the CL emission. The inhibition of CL intensity can be used to determine these substances at sub-micromolar levels.

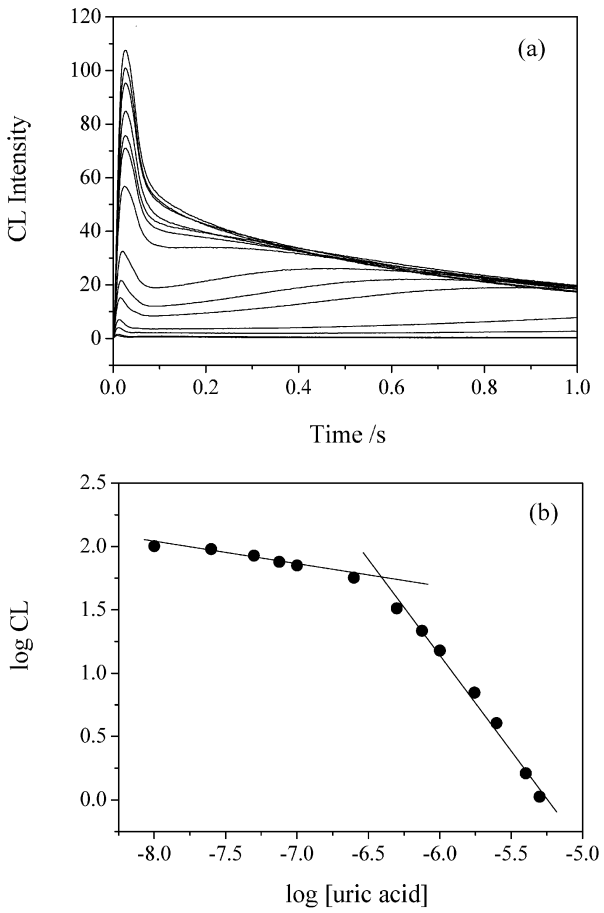

Fig. 8. (a) Stopped-flow CL intensity vs. time profiles for the reactions of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol, 1 M carbonate (pH 10.0) at various concentrations of uric acid (from top to bottom: 0, 0.01, 0.025, 0.05, 0.075, 0.10, 0.25, 0.50, 0.75, 1.00, 1.75, 2.50, 4.00, and 5.00 μM). (b) The double-log plot of peak CL intensity vs. the concentration of uric acid.

Table 1. Parameters for the double-log plots of CL intensity (y) versus concentration of reagent (x) obtained from the data in Fig. 9

Reagent	Linear equations	Dynamic range (M)	R^2
Uric Acid	y = -0.1834x + 0.5653	$1.0 \times 10^{-8} \sim 2.5 \times 10^{-7}$	0.9445
	y = -1.4984x – 7.8501	$5.0 \times 10^{-7} \sim 5.0 \times 10^{-6}$	0.9885
Ascorbic acid	y = -0.2692x + 0.033	$5.0 \times 10^{-8} \sim 5.0 \times 10^{-7}$	0.9475
	y = -1.718x – 8.9534	$7.5 \times 10^{-7} \sim 5.0 \times 10^{-6}$	0.9811
Acetaminophen	y = -0.2434x + 0.3364	$1.0 \times 10^{-7} \sim 1.0 \times 10^{-6}$	0.9288
p-Hydroxyphenyl	y = -0.0616x + 1.5726	$1.0 \times 10^{-7} \sim 2.5 \times 10^{-6}$	0.9410
acetic aicd	y = -1.2320x – 4.8548	$5.0 \times 10^{-6} \sim 1.0 \times 10^{-4}$	0.9646

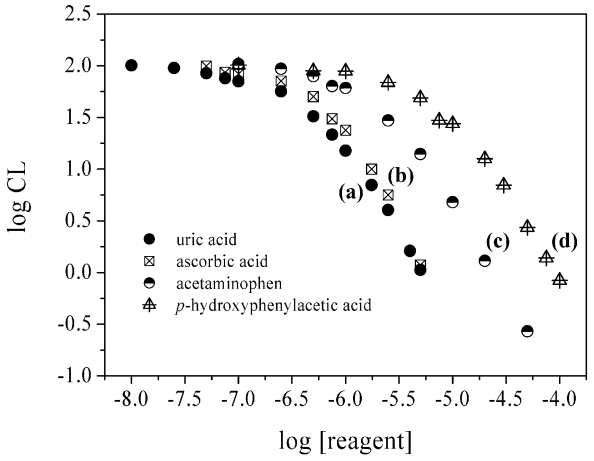

Fig. 9. The double-log plots of peak CL intensities vs. the concentrations of (a) uric acid (b) ascorbic acid (c) acetaminophen, and (d) *p*-hydroxyphenyl acetic acid. The CL intensities were measured for the reactions of 10 μM Co(II), 10 μM H_2O_2, 5 μM luminol, 1 M carbonate (pH 10.0) at various concentrations of each reagent.

ACKNOWLEDGMENT

The authors thank the National Science Council of the ROC for financial support (NSC 92-2113-M-002-032).

Received February 21, 2005.

REFERENCES

1. Robards, K.; Worsfold, P. J. *Anal. Chim. Acta* **1992**, *266*, 147.
2. Roda, A.; Pasini, P.; Guardigli, M.; Baraldini, M.; Musiani, M.; Mirasoli, M. *Fresenius J. Anal. Chem.* **2000**, *366*, 752.
3. Dodeigne, C.; Rhunus, L.; Lejeune, R. *Talanta* **2000**, *51*, 415.
4. Kubo, H.; Toriba, A. *Anal. Chim. Acta* **1997**, *353*, 345.
5. Jones, P.; Scowen, N. R. *Photochem. Photobiol.* **1987**, *45*, 283.
6. Nakamura, M.; Nakamura, S. *Free Rad. Biol. Med.* **1998**, *24*, 537.
7. Dapkevicius, A.; van Beek, T. A.; Niederlander, H. A. G.; de Groot, A. *Anal. Chem.* **1999**, *71*, 736.
8. Haqqani, A. S.; Sandhu, J. K.; Birnboim, H. C. *Anal. Biochem.* **1999**, *273*, 126.
9. Easton, P. M.; Simmonds, A. C.; Rakishev, A.; Egorov, A. M.; Candeias, L. P. *J. Am. Chem. Soc*. **1996**, *118*, 6619.
10. Kricka, L. J.; Ji, X. *Talanta* **1997**, *44*, 1073.
11. Navas Diaz, A.; Garcia Sanchez, F.; Gonzalez Garcia, J. A. *J. Photochem. Photobiol. A: Chemistry* **1995**, *87*, 99.
12. Gaikwad, A.; Silva, M.; Perez-Bendito, D. *Anal. Chim. Acta* **1995**, *302*, 275.
13. Lan, Z.-H.; Mottoal, H. A. *Analyst* **1999**, *121*, 211.
14. Chang, C. A.; Patterson, H. H. *Anal. Chem.* **1980**, *52*, 653.
15. Xiao, C.; King, D. W.; Palmer, D. A.; Wesolowski, D. J. *Anal. Chim. Acta* **2000**, *415*, 209.
16. Wang, J.-S.; Back, H. K.; Van Wart, H. E. *Biochem. Biophys. Res. Commun.* **1991**, *179*, 1320.
17. Yeh, H.-C.; Lin, W.-Y. *Talanta* **2003**, *59*, 1029.
18. Garcia-Campana, A. M.; Baeyens, W. R. G., Ed.; *Chemiluminescence in Analytical Chemistry*; Marcel Dekker, Inc.: New York, 2001; pp. 123-139.
19. Liu, Y.-M.; Liu, E.-B.; Cheng, J.-K. *J. Chromatogr. A* **2001**, *939*, 91.
20. Zhang, S.; Wu, Y.; Li, H. *Talanta* **2000**, *53*, 609.
21. Yuan, J. A.; Shiller, M. *Anal. Chem.* **1999**, *71*, 1975.
22. Wildes, P. D.; White, E. H. *J. Am. Chem. Soc.* **1973**, *95*, 26107.
23. Jansen, E. G. *Acc. Chem. Res.* **1971**, *4*, 31.
24. Mason, R. P.; Knecht, K. T. *Methods Enzymol.* **1994**, *233*, 112.
25. Merenyi, G.; Lind, J.; Eriksen, T. E. *J. Phys. Chem.* **1984**, *88*, 2320.
26. Lind, J.; Merenyi, G.; Eriksen, T. E. *J. Am. Chem. Soc.* **1983**, *105*, 7655-7661.