Abstract

Peroxynitrous acid (ONOOH) was formed by the on-line rapid reaction of acidified hydrogen peroxide with nitrite in a simple flow system. A weak chemiluminescent (CL) signal was observed due to the production of singlet oxygen (1O₂) when ONOOH reacted with NaOH, whereas the replacement of NaOH by Na₂CO₃ markedly enhanced the CL intensity. The predominant CL-enhanced pathway was achieved by the carbonate-catalyzed decomposition of peroxynitrite (ONOO⁻). Carbonate species was regenerated in the process, that is, carbonate acts as a catalyst. Based on the studies of CL and fluorescence spectra, a possible CL mechanism from the reaction of carbonate with ONOOH was proposed. In brief, ONOOH was an unstable compound in acidic solution and could be quenched into ONOO⁻ in basic media. It was suggested that ONOO⁻ reaction with excess HCO₃⁻ proceeded via one-electron transfer to yield bicarbonate ion radicals (HCO₃•). The recombination of HCO₃• may directly generate excited triplet dimers of two CO₂ molecules [(CO₂)₂*]. With the decomposition of this unstable intermediate to CO₂, the energy was released by CL emission. The addition of uranine into carbonate solution caused enhancement of the CL signal, which was due to a part of excited triplet dimers of two CO₂ molecules energy to transfer to uranine, resulting in two CL peaks.

Keywords: Peroxynitrite; (CO₂)₂*; Carbonate catalysis; Chemiluminescence decomposition

1. Introduction

Peroxynitrite (ONOO⁻) and its conjugate peroxynitrous acid (ONOOH) are powerful oxidants and capable of oxidizing a variety of biomolecules [1–3], which are receiving increasing attention as potential pathogenic agents in human disease [4,5]. ONOO⁻ is a relatively stable compound in alkaline solution (pKₐ of 6.8); however, once protonated to form ONOOH, it rapidly isomerizes to form nitric acid or decomposes along the weak O–O bond, with a half-life of less than 1 s at physiological pH and 37°C [6,7].

There are many reported methods for the synthesis of ONOO⁻ [8]. However, ONOO⁻ produced in the literature may contain decomposition products due to the off-line procedure, which makes the research results more complicated and difficult to interpret. Therefore, it is of great importance to be able to synthesize ONOO⁻ on-line. Saha et al. [8] reported a way of synthesizing ONOO⁻ involving the mixing of acidified hydrogen peroxide with nitrite in a simple flow system, and converting ONOOH to ONOO⁻ under alkaline condition, but the synthesized ONOO⁻ need be kept at −20°C for subsequent experiments. Based on the above study, we here synthesized ONOOH on-line and simultaneously investigated its chemiluminescence (CL) decomposition mechanism with carbonate solution by a flow method.

It is well known that carbonate is a major constituent in the physiological environment, and its total concentration is approximately 25 mmol l⁻¹ [9]. ONOO⁻ is unstable in the presence of carbonate solution [10,11]. However, the mechanism of the reaction of ONOO⁻ with carbonate solution remains to be a controversial subject [12–16]. For example, Radi et al. [12] proposed that the reaction of bicarbonate with ONOO⁻−enhanced luminol CL, possibly via the formation of ONOOCO₂⁻ intermediate. Whereas Hurst and co-worker [15] suggested that ONOOCO₂⁻ is formed as a result of the reaction of ONOO⁻ with CO₂, rather than the reaction of ONOOH with bicarbonate. Therefore, it is crucial to clarify the reaction mechanism of ONOO⁻ with carbonate solution.
In this study, we found, for the first time, a strong CL emitted from the reaction of on-line produced ONOOH with carbonate without any special CL reagents, such as luminol and lucigenin. It was demonstrated that carbonate was re-generated during the ONOOH carbonate reaction, i.e., carbonate species acts as a true catalyst. Based on the studies of CL and fluorescence spectra, a reasonable mechanism responsible for the CL phenomenon was discussed in detail.

2. Experimental

A flow system was designed for the CL spectra study. It consisted of two peristaltic pumps (SJ-1211; Atto, Tokyo, Japan), a F-2500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan). In brief, 0.02 mol l\(^{-1}\) H\(_2\)O\(_2\) in 0.1 mol l\(^{-1}\) HCl, 0.1 mol l\(^{-1}\) NO\(_2\)\(^-\) and 0.4 mol l\(^{-1}\) Na\(_2\)CO\(_3\) solutions or 0.4 mol l\(^{-1}\) Na\(_2\)CO\(_3\)-5×10\(^{-2}\) mol l\(^{-1}\) uranine were fed through separate lines into a cell placed inside the cell holder of the fluorescence spectrophotometer. The flow rates for H\(_2\)O\(_2\)-HCl, NO\(_2\)\(^-\) and Na\(_2\)CO\(_3\) solutions were 3.0, 3.0 and 3.5 ml/min, respectively. The excitation lamp was off and the emission slit width was opened maximally to 20 nm during the CL spectra recording. A Shimadzu UV-2401 UV-Vis recording spectrophotometer (Shimadzu, Kyoto, Japan) was used for measuring optical spectra.

All reagents were of analytical grade and used without further purification. Water was obtained from Milli-Q purification system (Barstad Thermoyne, USA). A 0.1 mol l\(^{-1}\) nitrite solution was prepared by dissolving 0.69 g NaNO\(_2\) (Beijing Chemical Reagent Company, Beijing, China), pre-dried at 110 °C for 4 h, in 100 ml of water. A small amount of sodium hydroxide (NaOH) was added to the above solution to prevent its decomposition and 1.0 ml of chloroform to inhibit bacterial growth. A mixing working solution of 0.02 M hydrogen peroxide (H\(_2\)O\(_2\)) and 0.1 M HCl was freshly prepared by volumetric dilution of commercial 30% (v/v) H\(_2\)O\(_2\) into the H\(_2\)O\(_2\)-HCl-NO\(_2\)\(^-\) solution. On the other hand, the replacement of H\(_2\)O by NaOH solution gave a weak CL emission (Experiment 2). It was due to the production of \(^1\)O\(_2\) (a well-known CL emitter). When comparing Experiments 2 and 3, the addition of Na\(_2\)CO\(_3\) led to a significant increase in the CL intensity. The injection of Na\(_2\)CO\(_3\)-uranine mixing solution into H\(_2\)O\(_2\)-HCl-NO\(_2\)\(^-\) solution (Experiment 4) provided the strongest CL emission. Therefore, the effect of carbonate on the CL signal was obvious. It was well known that a number of CL analyses were carried out in the presence of carbonate solution [17,18], not only based on its buffer function but also its CL enhancement effect. To clarify the CL mechanism of the present system, the following experiments were carried out.

3. Results and discussion

3.1. A batch method

In order to obtain the highest CL signal, the various mixing orders of reagents were measured by a batch method. In Table 1, Experiment 1 showed that there was no CL generation by the injection of 100 μl of H\(_2\)O solution into H\(_2\)O\(_2\)-HCl-NO\(_2\)\(^-\) solution. On the other hand, the replacement of H\(_2\)O by NaOH solution gave a weak CL emission (Experiment 2). It was due to the production of \(^1\)O\(_2\) (a well-known CL emitter). When comparing Experiments 2 and 3, the addition of Na\(_2\)CO\(_3\) led to a significant increase in the CL intensity. The injection of Na\(_2\)CO\(_3\)-uranine mixing solution into H\(_2\)O\(_2\)-HCl-NO\(_2\)\(^-\) solution (Experiment 4) provided the strongest CL emission. Therefore, the effect of carbonate on the CL signal was obvious. It was well known that a number of CL analyses were carried out in the presence of carbonate solution [17,18], not only based on its buffer function but also its CL enhancement effect. To clarify the CL mechanism of the present system, the following experiments were carried out.

| Experiment | CL system* | Relative CL intensity* |
|------------|------------|------------------------|
| 1          | H\(_2\)O    | 0                      |
| 2          | NaOH       | 0.01 ± 0.005           |
| 3          | Na\(_2\)CO\(_3\) | 9.8 ± 0.5             |
| 4          | Na\(_2\)CO\(_3\) + uranine | 230 ± 4.1              |

*The concentrations of H\(_2\)O\(_2\), HCl, NO\(_2\)\(^-\), NaOH and Na\(_2\)CO\(_3\) were 0.02, 0.1, 0.1, 0.15 and 0.4 M, respectively. The flow injection method was used. The flow rates of H\(_2\)O\(_2\)-HCl and NO\(_2\)\(^-\) solution were 3.0 ml/min. The Na\(_2\)CO\(_3\) solution flow rate was 3.5 ml/min.

* Each CL intensity represented the mean of five measurements ± standard deviation (S.D.).

3.2. Effects on O\(_2\) and CO\(_2\) in solution on CL

In aqueous solutions containing carbonate, small amounts of aqueous CO\(_2\) and O\(_2\) are usually produced at the mixing boundary. To investigate the effects of CO\(_2\) and O\(_2\) on CL signal, all solutions were prepared with fresh, ultrapure water and diluted to low concentration before use.

3.3. CL and fluorescent spectra

In order to identify the emitting species in this system, CL and fluorescent spectra were also measured. The CL spectrum from the reaction of the H\(_2\)O\(_2\)-HCl-NO\(_2\)\(^-\) and Na\(_2\)CO\(_3\) is shown in Fig. 1a, and it can be seen that the maximum of the CL spectrum is located at 443 nm. There are many reports about the decomposition of peroxynitrite to form singlet oxygen (\(^1\)O\(_2\)) [6,19,20], which is the light-emitting species. It is known that there are several maxima in the emission spectra of \(^1\)O\(_2\), i.e., 1269, 762, 634, 476,
Fig. 1. CL spectra of H$_2$O$_2$–HCl–NO$_2^−$ reaction in Na$_2$CO$_3$ (a) and Na$_2$CO$_3$–uranine (b) solutions. The concentrations of H$_2$O$_2$, HCl, NO$_2^−$, Na$_2$CO$_3$ and uranine were 0.02, 0.1, 0.1, 0.4 and 5 × 10$^{−7}$ mol l$^{−1}$, respectively. The flow injection method was used. The flow rates of H$_2$O$_2$–HCl solution and NO$_2^−$ solution were 3.0 ml/min. The Na$_2$CO$_3$ solution flow rate was 3.5 ml/min. The excitation lamp was off and the emission slit width was set at 20 nm.

and 381 nm [21]. Two quenchers of 1$^1$O$_2$, 1,4-diazabicyclo[2.2.2]octane (DABCO), and NaN$_3$ [22] were used in the present experiment. The results showed that DABCO and NaN$_3$ did not quench the CL intensity, which provided strong evidence that 1$^1$O$_2$ did not contribute to the observed CL. A number of investigations [17,18] have demonstrated that carbonate was a luminous species when present with a strong oxidant in basic solution. In our previous work [23], the CL emission band at 436–446 nm was also recorded (as shown in Fig. 1a), which was found to result from the decomposition of excited triplet dimers of two CO$_2$ molecules ([CO$_2$]$_2^*$) to carbon dioxide. Therefore, the CL observed in the present system was likely also due to the formation of [CO$_2$]$_2^*$.

As shown in Fig. 1b, when uranine solution was added into H$_2$O$_2$–HCl–NO$_2^−$–Na$_2$CO$_3$ flow system, the CL spectrum had two peaks with maximum located at 443 and 534 nm, respectively. Importantly, the first CL peak (443 nm) was the same as that obtained from the reactions of the H$_2$O$_2$–HCl–NO$_2^−$–Na$_2$CO$_3$ solutions (Fig. 1a).

The CL intensity at 443 nm, however, was significantly lowered after the addition of uranine. This phenomenon can be attributed to the transfer of energy from the excited triplet dimers of two CO$_2$ molecules to uranine. In order to record simultaneously two CL peaks with maximum located at 443 and 534 nm, respectively, the concentration of uranine was diluted to 5 × 10$^{−7}$ mol l$^{−1}$ with 0.4 mol l$^{−1}$ carbonate solution. The CL peak at 534 nm was found to arise from uranine, likely as a result of the chemical excitation of uranine by the triplet dimers ([CO$_2$]$_2^*$). The fluorescence spectra of uranine in Na$_2$CO$_3$ solution with and without the addition of H$_2$O$_2$–HCl–NO$_2^−$ solutions have also been recorded. The results showed that the fluorescent maximum wavelength of uranine in Na$_2$CO$_3$ solution was located at 534 nm, which was the same as CL emission maximum of uranine. When adding uranine–Na$_2$CO$_3$ solution into H$_2$O$_2$–HCl–NO$_2^−$ solution, the fluorescent maximum was situated at 534 nm (Fig. 2), and the fluorescent intensity of uranine remained constant, which indicated that uranine was not destroyed during the CL reaction, and [CO$_2$]$_2^*$ was a CL emitter and uranine was only a sensitizer.

3.4. UV spectrum

The UV absorption of peroxynitrous acid from the reaction of nitrite and H$_2$O$_2$/HCl was given in Fig. 3. The absorption peak at 301 nm was due to the formation of nitrate by the isomerization of peroxynitrous acid (solid line). However, after rapid mixing of HOONO with carbonate solution...
C. Lu, J.-M. Lin / Catalysis Today 90 (2004) 343–347

Fig. 3. Absorption spectra of peroxynitrous acid from the reaction of nitrite and H2O2–HCl in the absence (solid line) and presence (dashed line) of Na2CO3 solution. The concentrations of NO2−, H2O2, HCl and Na2CO3 were 0.6, 0.7, 0.6 and 1.0 mol l−1, respectively. The volume of each solution was 1.0 ml.

(dashed line), a new band appeared at 354 nm resulted from the recombination between •NO2 radicals to form nitrite, whereas the characteristic band of nitrate at 301 nm did not disappear. This suggested that nitrate [5,8] is the only product in the spontaneous decomposition of peroxynitrous acid (solid line) and both nitrite and nitrate [4,16] were formed simultaneously in the reaction from peroxynitrous acid with carbonate solution (dashed line).

3.5. CL mechanism from carbonate-catalyzed peroxynitrite decomposition

The mechanism of carbonate-catalyzed ONOO− chemiluminescence decomposition can be deduced from our above observations as follows.

HOONO is formed from the reaction of nitrite with acidified H2O2 (Reaction (1)), and it is an unstable compound in acidic solution and can be converted into ONOO− in basic solution as a function of the protonation of CO32− to HCO3− according to Reactions (2) and (3):

H2O2 + HNO2 → HOONO + H2O (1)

CO32− + H2O ⇌ HCO3− + OH− (2)

ONOOH + OH− → ONOO− + H2O (3)

It is well-known that ONOO−/•NO2 and HCO3•/HCO3− couples have similar one-electron standard redox potentials (E0(HCO3•/HCO3−) = +1.5 V, E0(ONOO−/•NO2) = +1.5 V) [12]. Therefore, when ONOO− reacts with excess HCO3−, one-electron oxidation of HCO3− by ONOO− is thermodynamically favorable to yield bicarbonate radicals (HCO3•) according to Reaction (4) [5,12]:

ONOO− + HCO3− + H+ → HCO3• + •NO2 + OH− (4)

As spectrophotometrically experimental data were shown in Fig. 3, the recombination between •NO2 radicals can generate nitrite and nitrate in aqueous solution [4,16]:

•NO2 + •NO2 + H2O → NO2− + NO3− + 2H+ (5)

The recombination of HCO3• could generate excited triplet dimers of two CO2 molecules ([CO2]2) directly [24]:

2HCO3• → [CO2]2 + H2O2 (6)

With the decomposition of this unstable intermediate to CO2, the energy is released [23,25]:

(CO2)2∗ → 2CO2 + hν (λmax = 443 nm) (7)

In alkaline solution dissolved CO2 is in fast equilibrium with carbonate and therefore carbonate was regenerated during the ONOOH–carbonate reaction, i.e., carbonate species acts as a true catalyst.

The decomposition energy of (CO2)2∗ was calculated by the extended Hückel molecular orbital (EHMO) method and found to be 132 kcal/mol [26], which is high enough to excite uranine (55.5 kcal/mol) [21]:

(CO2)2∗ + uranine → 2CO2 + uranine∗ (8)

uranine∗ → uranine + hν (λmax = 534 nm) (9)

The CL wavelength at 534 nm (Fig. 1b) can be considered as a result of intramolecular energy transfer in that the part of energy from the excited triplet dimers of two CO2 molecules is transferred to uranine. Therefore, the CL spectrum of H2O2–HCl–NO2−–Na2CO3–urate solutions shows two bands.

4. Conclusion

We have demonstrated that the decomposition of peroxynitrite in the presence of carbonate generates excited triplet dimers of two CO2 molecules ([CO2]2) and the energy is released by CL with the decomposition of this unstable intermediate to CO2. In alkaline solution produced CO2 is in fast equilibrium with carbonate and therefore carbonate was
regenerated from the reaction of ONOOH with carbonate reaction, i.e., carbonate species acts as a catalyst.

Acknowledgements

Financial support from National Science Fund for Distinguished Young Scholars of China (No. 20125514) and from National High Technology Research and Development Program of China (863 Program) (No. 2001AA635030) is gratefully acknowledged.

References

[1] R.M. Uppu, G.L. Squadrito, W.A. Pryor, Arch. Biochem. Biophys. 327 (1996) 335.
[2] S. Goldstein, G. Czapski, J. Am. Chem. Soc. 120 (1998) 3458.
[3] L. Zhu, C. Gunn, J.S. Beckman, Arch. Biochem. Biophys 298 (1992) 452.
[4] S.V. Lymar, Q. Jiang, J.K. Hurst, Biochemistry 35 (1996) 7855.
[5] A. Denicola, B.A. Freeman, M. Trujillo, R. Radi, Arch. Biochem. Biophys. 333 (1996) 49.
[6] A.U. Khan, D. Korzicz, A. Kolbanovskiy, M. Desai, K. Frenkel, N.E. Geacintov, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 2984.
[7] J.R. Mahoney, J. Am. Chem. Soc. 92 (1970) 5262.
[8] A. Saha, S. Goldstein, D. Cabelli, G. Czapski, Free Radic. Biol. Med. 24 (1998) 653.
[9] A. Gou, D. Dutta, S.R. Thom, H. Ichinose, Arch. Biochem. Biophys. 335 (1996) 42.
[10] S. Goldstein, G. Czapski, Free Radic. Biol. Med. 19 (1995) 515.
[11] J.N. Lemercier, S. Padmaja, R. Curtis, G.L. Squadrito, R.M. Uppu, W.A. Pryor, Arch. Biochem. Biophys. 345 (1997) 160.
[12] R. Radi, T.P. Cregg, J.S. Beckman, B.A. Freeman, Biochem. J. 290 (1993) 51.
[13] G. Mentiyi, J. Lind, S. Goldstein, J. Am. Chem. Soc. 124 (2002) 40.
[14] G.R. Hodges, K.U. Ingold, J. Am. Chem. Soc. 121 (1999) 10695.
[15] S.V. Lymar, J.K. Hurst, J. Am. Chem. Soc. 117 (1995) 6887.
[16] S.V. Lymar, J.K. Hurst, Inorg. Chem. 37 (1998) 294.
[17] J.-M. Lin, T. Hoho, Anal. Chem. Acta 323 (1996) 60.
[18] D. Stawinska, J. Stawinski, J. Biolumin. Chemilumin. 13 (1998) 13.
[19] P. Di Mascio, E.J.H. Bechara, M.H.G. Mediros, K. Birivba, H. Sies, FEBS Lett. 355 (1994) 287.
[20] A.U. Khan, J. Biolumin. Chemilumin. 10 (1995) 329.
[21] X.-Z. Wu, M. Yamada, T. Hoho, S. Suzuki, Anal. Chem. 61 (1989) 1305.
[22] J.-M. Lin, M. Yamada, Anal. Chem. 72 (2000) 1148.
[23] J.-M. Lin, M. Yamada, Anal. Chem. 71 (1999) 1760.
[24] J. Wierzchowski, D. Stawinska, J. Stawinski, Z. Phys. Chem. Neue Folge 148 (1986) 197.
[25] H.F. Coombs, H.P. Richter, C.A. Hellex, J. Am. Chem. Soc. 91 (1969) 7209.
[26] L.J. Bollyky, J. Am. Chem. Soc. 92 (1970) 3230.