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Time-Resolved Luminol Chemiluminescence for Simultaneous Determination of Cobalt(II) and Copper(II)

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The peroxidation of luminol was performed using both Co(II) and Cu(II) as a catalyst in the presence of cysteine. A luminol chemiluminescence (CL) catalyzed by Co(II) occurred instantly from the initiation of the reaction, whereas a Cu(II)-catalyzed luminol CL suddenly appeared after a dark period. The time-resolved luminol CL were applied for the simultaneous determination of Co(II) and Cu(II).

Many metal ions catalyse the oxidation of luminol with hydrogen peroxide (H_2O_2), producing chemiluminescence (CL) emission. The luminol CL has been employed for the determination of trace metal ions. However, it is usually difficult to determine simultaneously more than two metal ions using the conventional luminol CL, since the CL emission begins immediately after the initiation of the CL reaction in all metal ions.

We have previously reported that a CL flash suddenly appeared after a certain dark period from the initiation of the reaction in the Cu(II)-catalyzed oxidation of cysteine with oxygen in the presence of luminol.1) In the course of our subsequent studies, we have found that the CL response curve was time-resolved into two peaks corresponding to Co(II) and Cu(II) when the peroxidation of luminol was carried out using both Co(II) and Cu(II) as a catalyst in the presence of cysteine. The time-resolved CL reaction system can thus be applied for the simultaneous determination of Co(II) and Cu(II).

The general experimental procedure consisted of pipeting 0.5 cm^3 of a solution containing Co(II) and Cu(II) into a glass cuvette in a luminometer. The solution was saturated with oxygen by bubbling. Next, a 1 cm^3 portion of cysteine solution and a 1 cm^3 portion of a solution containing luminol and H_2O_2 were simultaneously injected into the cuvette. Thus the CL reaction was initiated and the light emission was detected by a photomultiplier tube. The resultant photocurrent was converted to a current and displayed on a chart recorder. The solution containing luminol and H_2O_2 was prepared with a 0.1 M (1 M = 1 mol dm^{-3}) NaOH solution. A cysteine solution was made daily. Bubbling of oxygen at 60 cm^3 min^{-1} and vigorous agitation by a magnetic stirrer were performed in each run. All CL measurements were made at 25 °C. The maximum light emission was referred to as the CL intensity. The time period from the reaction initiation to a CL flash was defined as the delay time.

The measurements of the conventional luminol CL were carried out according to the procedure in which a solution containing 2.5×10^{-3} M luminol and 3.0×10^{-4} M H_2O_2 was injected into a 3.0×10^{-8} M Co(II) solution or a 3.0×10^{-5} M Mn(II), Cr(III), Fe(III), Ni(II), Cu(II) or Zn(II) solution. Water was employed in place of a cysteine solution. Typical CL response curves are shown in Fig.1. The light emission reached a maximum within 10 s and then began to rapidly decay in all metal ions. The greatest CL intensity was obtained using Co(II) as a catalyst. The CL intensity observed in Co(II) was a few orders higher than that in other metal ions. These results suggest that only Co(II) could be selectively detected in concentrations below the detection limit of other metal ions.

Next, the Cu(II)-catalyzed oxidation of cysteine with H_2O_2 was

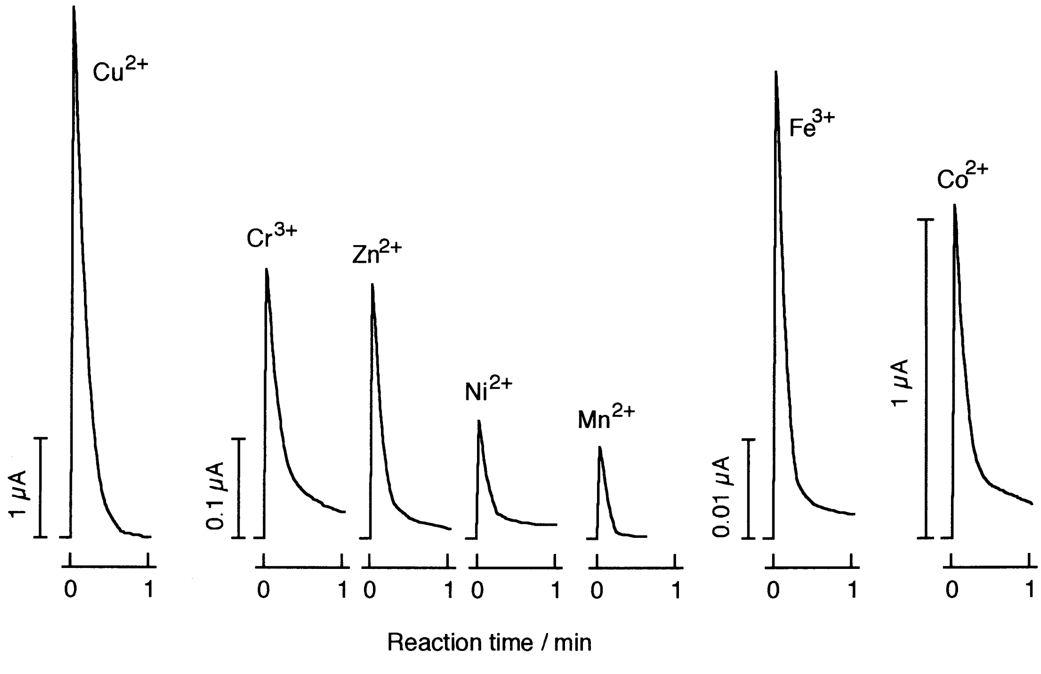

Fig.1. Typical CL response curves for the conventional luminol CL catalyzed by metal ions with H_2O_2.
metal ions: [Co(II)]=3.0×10^{-8} M, [Mn(II)], [Cr(III)], [Fe(III)], [Ni(II)], [Cu(II)], [Zn(II)]=3.0×10^{-5} M, [luminol]=2.5×10^{-3} M, [H_2O_2]=3.0×10^{-4} M.

performed in the presence of luminol according to the procedure in which a 2.0×10^{-5} M Cu(II) solution was used in place of a mixture of Co(II) and Cu(II). Typical CL response curve is shown in Fig.2 (curve 1). A CL flash suddenly appeared after a dark period of about 7 min from the initiation of the reaction.

We thus examined the peroxidation of luminol using both Co(II) and Cu(II) in the presence of cysteine according to the procedure in which a solution containing 2.0×10^{-5} M Cu(II) and 6.0×10^{-6} M Co(II) was used. Typical CL response curve is shown in Fig.2 (curve 2). Two peaks appeared in the CL response curve. The first, nearly instantaneous peak reached its maximal height within 10 s and then rapidly decayed. The maximal height of the second peak occurred after about 7 min. The CL intensity and delay time in the presence of Co(II) was about the same as that with Cu(II) alone. The first and second peak heights increased with increasing Co(II) and Cu(II) concentrations, respectively. Therefore, the former peak was due to Co(II) while that of the latter to Cu(II).

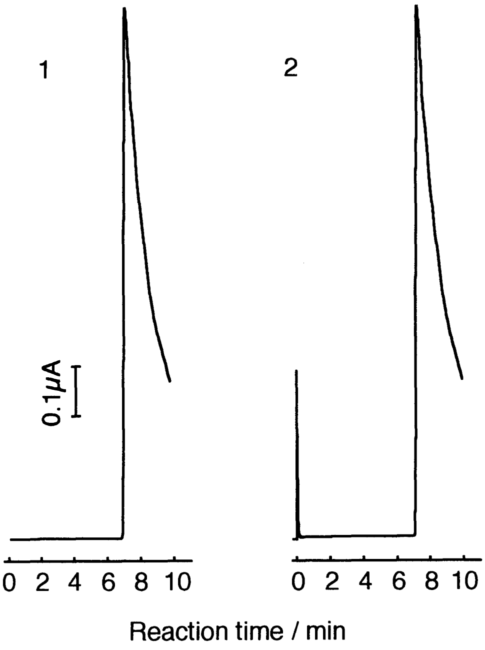

Fig.2. Luminol CL catalyzed by Cu (II) or both Cu(II) and Co(II). [luminol]=2.5×10^{-3} M, [H_2O_2]=1.0×10^{-3} M, [cysteine]=6.0×10^{-4} M.
1: [Cu(II)]=2.0×10^{-5} M,
2: [Cu(II)]=2.0×10^{-5} M,
[Co(II)]=6.0×10^{-6} M.

The appearence of the time-resolved CL could be explained as follows. The luminol CL reaction catalyzed by Co(II) with H_2O_2 added proceeds quickly after the initiation of the reaction.

$$\text{luminol} + H_2O_2 \xrightarrow{Co^{2+}} \text{aminophthalate} + N_2 + H_2O + h\nu$$

On the other hand, Cu(II) catalyses preferentially the catalytic oxidation of cysteine with oxyzen, resulting in the formation of H_2O_2 and cystine.

$$\text{cysteine} + O_2 \xrightarrow{Cu^{2+}} \text{cystine} + H_2O_2$$

During cysteine oxidation, Cu(II) is strongly complexed with cysteine, thereby being ineffective for the luminol CL reaction.[1] After complete oxidation of cysteine, the Cu(II)-catalyzed luminol CL reaction is subsequently commenced using accumulated H_2O_2, thus resluting the appearence of the second peak.

$$\text{luminol} + H_2O_2 \xrightarrow{Cu^{2+}} \text{aminophthalate} + N_2 + H_2O + h\nu$$

The CL intensity of each peak corresponding to Cu(II)[1] and Co(II) was remarkably lower than that observed in the conventional luminol CL. The decrease of the CL intensity of the first peak is probably attributable to the quenching effect of cysteine on the Co(II)-catalyzed luminol CL with H_2O_2, since antioxidant such as cysteine acts effectively as quenching agents for luminol radicals.[2] No time-resolved luminol CL was observed, when other metal ions were used in place of Co(II). These results suggest that the time-resolved luminol CL system could be applied for the simultaneous determination of Co(II) and Cu(II).

In subsequent studies, the optimum conditions for the simultaneous quantification of Co(II) and Cu(II) were determined by measuring the CL intensities, so as to be maximal for the second peak under optimum conditions. Analytical calibration curves for Co(II) and Cu(II) were thus prepared under the following optimized conditions: a 6.0×10^{-4} M cysteine solution and a solution containing 2.5×10^{-3} M luminol and 3.0×10^{-4} M H_2O_2 were simultaneously injected into a working solution containing both Co(II) and Cu(II). A pH value of the mixture was 12.5. The working solutions of Co(II) containing 2.0×10^{-5} M Cu(II) were made up, whereas those of Cu(II) containing 4.0×10^{-6} M Co(II) were also prepared. Logarithmic calibration curves of Co(II) and Cu(II) were linear over the range 5.0×10^{-7} to 1.0×10^{-5} M and 2.0×10^{-6} to 2.0×10^{-5} M with slopes of 0.71 and 1.23, respectively. Within these concentration ranges, the mutual interference on the CL intensity between Co(II) and Cu(II) was less than 3%. The relative standard deviation of five successive experiments was 2.5% at 5.0×10^{-6} M of Co(II) and 3.0% at 5.0×10^{-6} M of Cu(II).

In conclusion, the time-resolved luminol CL is potentially useful for the new indicator reaction for the simultaneous determination of Co(II) and Cu(II). Further studies on extension to real samples are under way.

References

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