CHEMISTRY LETTERS, pp. 843-846, 1990.

Chemiluminescence of L-tryptophan in Copper(II) - Hydrogen Peroxide - Potassium Sodium Tartrate System

Tadashi HARA,* Kazuhiko TSUKAGOSHI, Toshihito IMAI, Naoki YOSHIDA, and Riichiro NAKAJIMA

Department of Chemical Engineering, Faculty of Engineering, Doshisha University, Karasuma Imadegawa, Kamigyo-ku, Kyoto 602

L-tryptophan was found for the first time to show the chemiluminescence in the copper(II) - hydrogen peroxide - potassium sodium tartrate system with its maximum chemiluminescence intensity at about 3 h. Other α-amino acids such as L-histidine, L-methionine, and L-isoleucine showed no chemiluminescence. L-tryptophan in the concentration range of 1.0×10^{-5} - 1.0×10^{-3} mol dm^{-3} apparently showed the chemiluminescence corresponding to its concentration.

A new method using a chemiluminescence (CL) reaction has been established by the present authors for the determination of small amount of protein or α-amino acid.[1,2] The method is based on the lowering of the catalytic activity of a metal ion for the CL reaction in the presence of protein or α-amino acid. It was very sensitive, but a protein and an α-amino acid had to be indirectly measured by using a complex CL reaction system. However, there have been no reports in regard to the analytical method for α-amino acid by use of the CL from α-amino acid itself. During the course of such investigations on the new CL reaction which could be used for the simple and direct analysis of an α-amino acid, L-tryptophan was found to show the CL in the copper(II) - hydrogen peroxide - potassium sodium tartrate system. The present paper will describe the CL of L-tryptophan.

Deionized water was distilled for use. All α-amino acids were purchased from Kyowa Hakko Co. Ltd., and other reagents were of commercially available special grade. Copper(II) sulfate and iron(II) ammonium sulfate were used as Cu(II) and Fe(II) catalysts.

A schematic diagram of the apparatus used in the present study is shown in Fig. 1. The B solution (10 ml) [0.5 mol dm^{-3} hydrogen peroxide

(H_2O_2)] was injected into the A solution (pH 10.4) (40 ml) [1.0 × 10^{-4} mol dm^{-3} Cu(II), 1.0 × 10^{-2} mol dm^{-3} potassium sodium tartrate (PST), 0.1 mol dm^{-3} potassium hydroxide, 0.1 mol dm^{-3} boric acid, and 1.0 × 10^{-3} mol dm^{-3} α-amino acid] in a black box (E) by use of a syringe(F). Each reagent concentration indicates the concentration in a final reaction

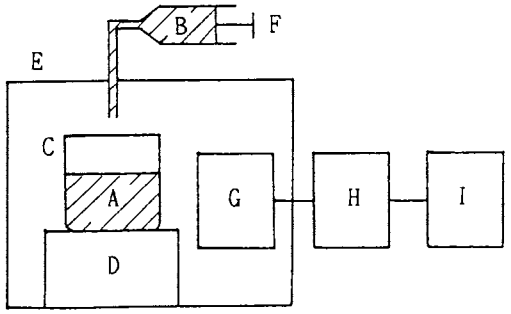

Fig. 1. Schematic diagram of the apparatus. A : A solution, B : B solution, C : reaction cell, D : stirrer, E : black box, F : syringe, G : photomultiplier, H : amplifier, and I : recorder.

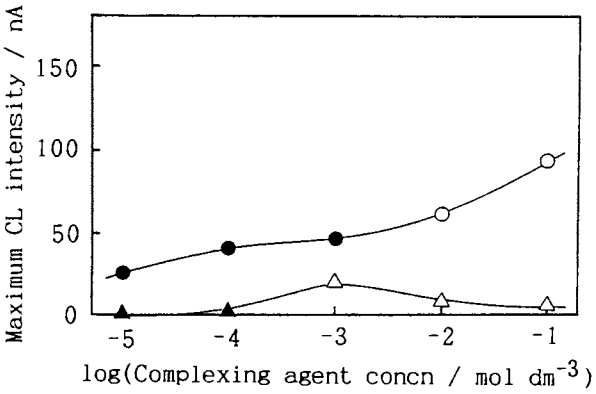

Fig. 2. Effect of complexing agent concn on the CL intensity of Cu(II) - H_2O_2 system. ○ : PST, △ : ST, and ● , ▲ : precipitate was observed after the reaction.
Conditions : 1.0 x 10^{-4} mol dm^{-3} Cu(II) , 5.0 x 10^{-2} mol dm^{-3} H_2O_2, 0.1 mol dm^{-3} H_3BO_3, and 0.1 mol dm^{-3} KOH.

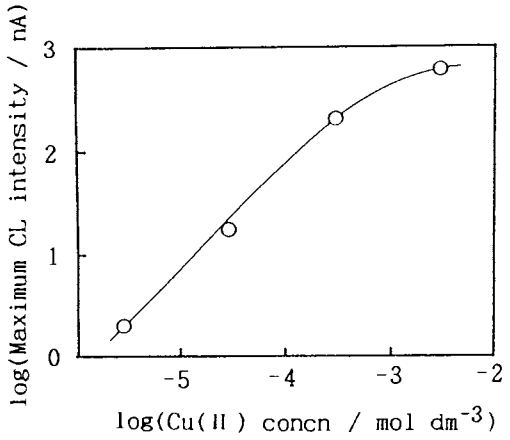

Fig. 3. Relationship between Cu(II) concn and maximum CL intensity.
Conditions : 4.0 x 10^{-1} mol dm^{-3} H_2O_2, 1.0 x 10^{-1} mol dm^{-3} PST, 0.1 mol dm^{-3} H_3BO_3, and 0.1 mol dm^{-3} KOH.

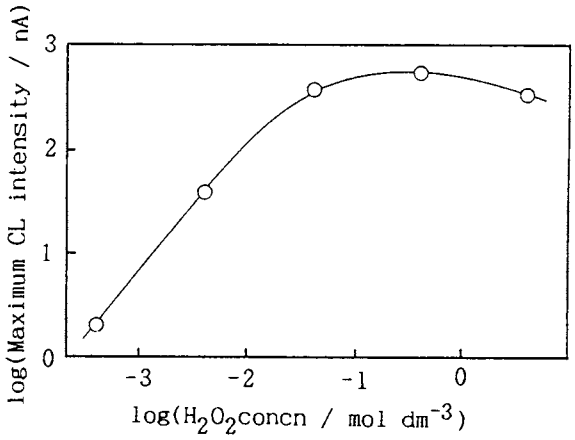

Fig. 4. Relationship between H_2O_2 concn and maximum CL intensity.
Conditions : 3.0 x 10^{-3} mol dm^{-3} Cu(II) , 1.0 x 10^{-1} mol dm^{-3} PST, 0.1 mol dm^{-3} H_3BO_3, and 0.1 mol dm^{-3} KOH.

solution (50 ml) after the A and B solutions were mixed. The CL of the reaction solution in a reaction cell (C) was measured by a photomultiplier (G) (Hamamatsu Photonics, R931), amplified with an amplifier (H) (Horiba Ltd., OPE-402), and recorded on a recorder (I) (Yokogawa Electric Works, Ltd., 3046).

The Cu(II) - H_2O_2 - PST system (System-1) or the Cu(II) - H_2O_2 - sodium tripolyphosphate (ST) system showed the CL in the absence of CL reagents such as luminol and 1,10-phenanthroline (Fig. 2). The maximum CL intensity in the System-1 increased with an increasing concentration of Cu(II) or H_2O_2 (Figs. 3 and 4). The maximum CL intensities which were observed in all these experiments were obtained within 5 min after a H_2O_2 solution was added.

The CL in the System-1 was examined in the presence of each α-amino acid such as L-tryptophan, L-histidine, L-methionine, and L-isoleucine, where the same concentration of the above-mentioned value was used. The CL intensity was enhanced in the presence of L-tryptophan, while no CL was

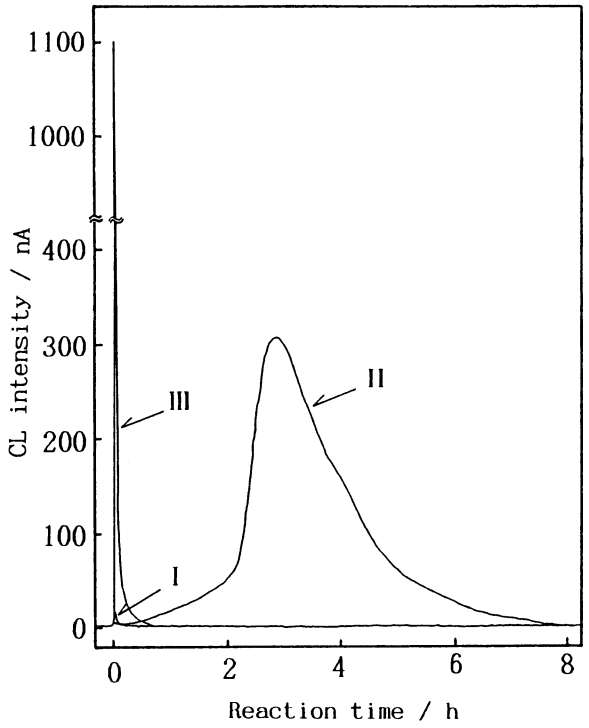

Fig. 5. Chemiluminescence of L-tryptophan and 1,10-phenanthroline in the Cu(II) - H_2O_2 - PST system.
I : 1.0×10^{-4} mol dm^{-3} Cu(II), 1.0×10^{-2} mol dm^{-3} PST, 0.1 mol dm^{-3} H_3BO_3, 0.1 mol dm^{-3} KOH, and 0.5 mol dm^{-3} H_2O_2.
II : 1.0×10^{-4} mol dm^{-3} Cu(II), 1.0×10^{-2} mol dm^{-3} PST, 1.0×10^{-3} mol dm^{-3} L-tryptophan, 0.1 mol dm^{-3} H_3BO_3, 0.1 mol dm^{-3} KOH, and 0.5 mol dm^{-3} H_2O_2.
III : 1.0×10^{-4} mol dm^{-3} Cu(II), 1.0×10^{-2} mol dm^{-3} PST, 1.0×10^{-5} mol dm^{-3} 1,10-phenanthroline, 0.1 mol dm^{-3} H_3BO_3, 0.1 mol dm^{-3} KOH, and 0.5 mol dm^{-3} H_2O_2.

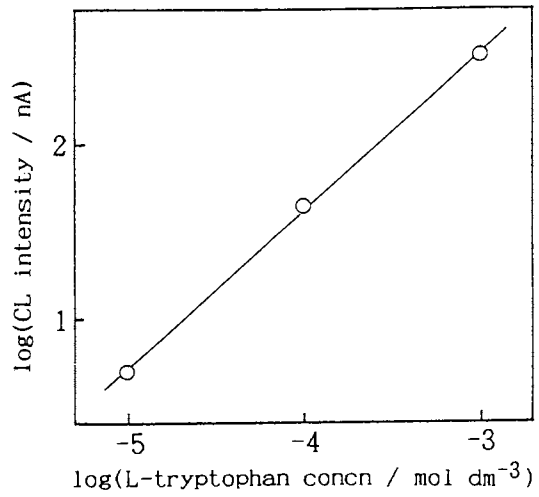

Fig. 6. Relationships between L-tryptophan concn and Cl intensity.

observed in the presence of L-histidine, L-methionine, and L-isoleucine in the System-1. The CL curve (curve-II) in the presence of L-tryptophan in the System-1 was shown together with the CL curve (curve-III) in the presence of 1.0×10^{-5} mol dm^{-3} 1,10-phenanthroline in the System-1 (Fig.5), where 1,10-phenanthroline was used for comparison because it is known as a CL reagent in the Cu(II) - H_2O_2 system. The maximum CL intensity and CL peak area of curve-II were about 310 nA and 1030 nA h, while those of curve-III were about 1100 nA and 91 nA h.

Moreover, the effect of iron(II) on the catalytic activity of the System-1 was examined. L-tryptophan showed no CL within 6 h in the Fe(II) - H_2O_2 - PST system, but it showed the CL in Cu(II) - Fe(II) - H_2O_2 - PST system (System-2). The maximum CL intensity was observed within 1 h in the System-1 and at several hours in the System-2 after the initiation of the reaction. The CL obtained for L-tryptophan in the presence of L-histidine, L-methionine, and L-isoleucine in the System-2 approximately corresponded to the CL obtained for L-tryptophan alone. L-tryptophan in the concentration range of 1.0×10^{-5} - 1.0×10^{-3}mol dm^{-3} apparently showed the CL intensity corresponding to its concentration (Fig. 6).

As has been described above it has been elucidated that the CL from L-tryptophan in the Cu(II) - (Fe(II)) - H_2O_2 - PST system could be potentially applied to a sensitive and selective analysis of L-tryptophan.

References

1) T. Hara, M. Toriyama, T. Ebuchi, and M. Imaki, Chem. Lett., 1985, 341.
2) T. Hara, K. Tsukagoshi, and Y. Kurita, Bull. Chem. Soc. Jpn., 62, 1501 (1989).
3) O. S. Fedorova, S. E. Olkin, and V. M. Berdnikov, Z. Phys. Chem. (Leipzig), 263, 529 (1982).
4) M. Ishii, M. Yamada, and S. Suzuki, Bunseki Kagaku, 35, 373 (1986).

(Received January 17, 1990)