CHEMISTRY LETTERS, pp. 1373-1376, 1991.

Chemiluminescence of Tryptophan with Electrogenerated Tris(2,2'-bipyridine)ruthenium(III)

Kazuo UCHIKURA* and Makoto KIRISAWA
College of Pharmacy, Nihon University, Narashinodai, Funabashi, Chiba 274

A system for electrogenerated chemiluminescence based on the reaction of electrogenerated $Ru(bpy)_3^{3+}$ with tryptophan (Trp) is described. The orange chemiluminescence was generated by reaction of $Ru(bpy)_3^{3+}$ with Trp in acidic solution. The lowest detection limit was 0.1 pmol for Trp. This newly demonstrated system should prove useful for the selective and sensitive detection of Trp.

In our previous study, oxalate in urine was determined by flow-injection analysis using electrochemiluminescence (ECL) from oxalate-Tris(2,2'-bipyridine)ruthenium(II) ($Ru(bpy)_3^{2+}$).[1)] ECL corresponding to emission from $Ru(bpy)_3^{2+*}$ has been observed previously.[2)] Similar observations have also been made for the thermal reduction of $Ru(bpy)_3^{3+}$ with reducing agents such as hydralazine,[3)] alkali (OH^-),[4)] organic acids[5)] and alkylamines.[6)] Similarly, reactions of $Ru(bpy)_3^{1+}$ with oxidizing agents such as 10-methylphenothiazine radical cation[7)] and peroxydisulfate[8)] have been reported. In general, aromatic substituted amines such as aniline, diphenylamine and triphenylamine, bipyridinium ions such as paraquat, and nitroaromatics such as nitrobenzene have been found to generate no chemiluminescence at any of the pH values examined.[6)] In such a case, it was observed that one kind of aromatic substituted amine, Trp was reacted with $(bpy)_3^{3+}$ acidic aqueous solution to generate chemiluminescence. The present paper describes a new aqueous ECL system based on $Ru(bpy)_3^{2+}$ with Trp as a reducing agent.

All the reagents and solvents were of guaranteed grade. Water was deionized and distilled using a hard-glass vessel. Stock solutions of oxalate and indole derivatives were prepared at 10^{-2} mol dm^{-3} in water-methanol (1:1, v/v) and diluted with the carrier solution before use.

Chemiluminescence intensity was measured using a two-channel flow-injection system[1)] and a chemiuminescence detector (LC30-DPC10, Nihon Denshi Co., Japan). The electrochemical oxidation mode was for

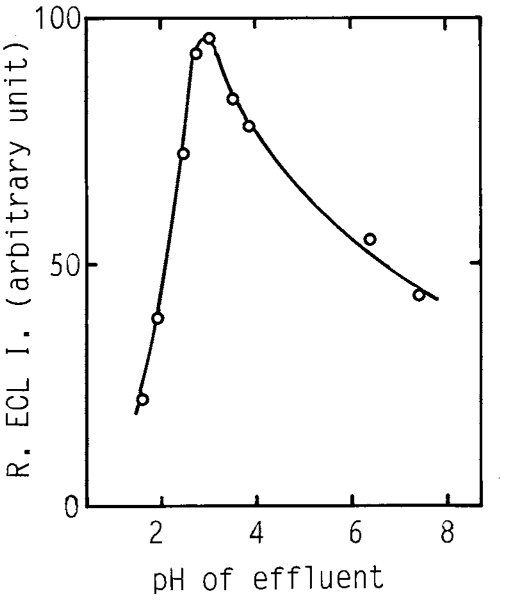

Fig. 1. Effect of effluent pH on ECL intensity. Trp: 40 pmol.

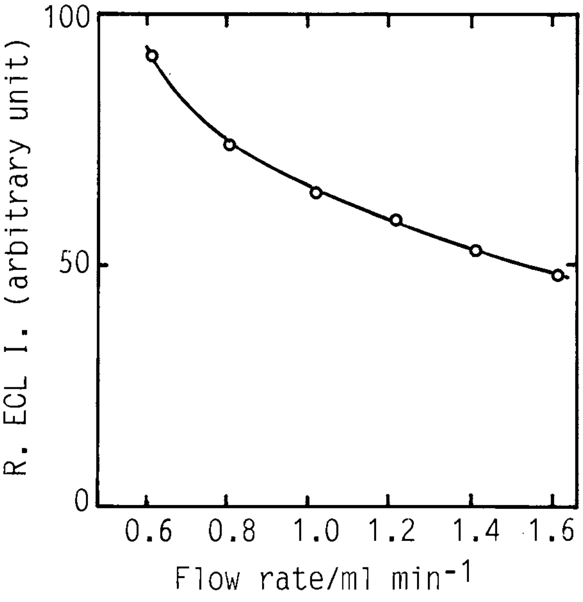

Fig. 2. Effect of flow rate on ECL intensity. Trp: 40 pmol.

controlled current electrolysis, and the current was maintained at 80 µA with a potentio-galvanostat (HA-101, Hokutodenkou, Japan). The carrier solution was a mixture of 10 mM phosphoric acid-acetonitrile (1:1, v/v). $Ru(bpy)_3^{2+}$ dichloride was obtained from Sigma (St. Louis, MO, USA). $Ru(bpy)_3^{2+}$ solution was dissolved at 0.24 mM in 10 mM H_2SO_4.

Immediately after addition of Trp to $Ru(bpy)_3^{3+}$ acidic solution, marked orange light was observed with a lifetime of less than one second. Preliminary studies on the selectivity, sensitivity, and analytical applicability of this chemiluminescence reaction for Trp and also its analogues were carried out

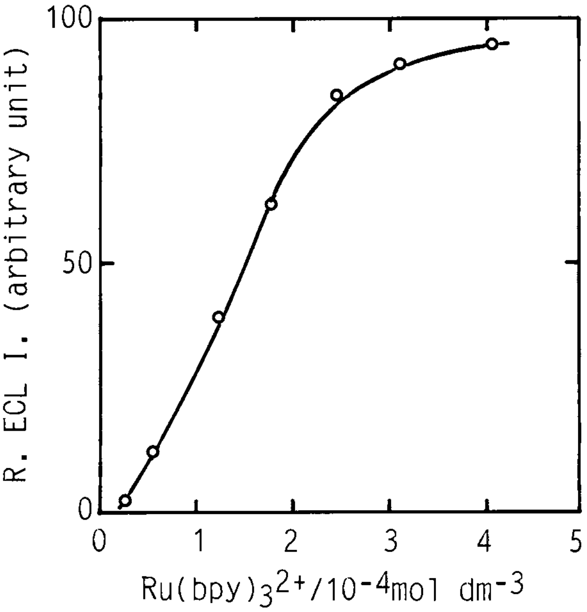

Fig. 3. Effect of $Ru(bpy)_3^{2+}$ concentration on ECL intensity. Trp: 40 pmol.

using the FIA system. Because of the flow electrochemical reactor (ECR)[10)] coupled in the reagent stream line in this FIA system, prior electrical oxidation of $Ru(bpy)_3^{2+}$ solution was not required, and continuous long-term operation was possible by supplementation with reagent solution. The factors affecting ECL production of Trp were examined. The effect of the effluent pH is shown in Fig. 1. The intensity was maximal at pH 3. The effect of flow rate on the ECL intensity was examined over the range 0.6-1.6 ml min^{-1}. The intensity decreased slightly with increasing flow rate (Fig. 2). Further, the effect of $Ru(bpy)_3^{2+}$ concentration on ECl intensity is shown in Fig. 3. ECL intensity was constant above 0.25 mM $Ru(bpy)_3^{2+}$.

Table 1. Relative ECL intensity of 3-substituted indole derivatives

Compound	Substituent	Relative ECL intensity
1	H	12
2	CHO	2
3	$OCOCH_3$	15
4	COOH	1
5	CH_2COOH	16
6	$CH_2COCOOH$	15
7	CH_2CH_2OH	3
8	$CH_2CH(OH)COOH$	2
9	$CH_2CH(NH_2)COOH$	100
10	$CH_2CH_2NH_2$	3
11	$CH_2CH(NH_2)COOC_2H_5$	4

Table 2. Relative ECL intensity of 3-, 5-disubstituted indole derivatives

Compound	Substituent		Relative ECL intensity[a)]
	5-	3-	
1	OH	CH_2COOH	2
2	OH	$CH_2CH_2NH_2$	2
3	OH	$CH_2CH(NH_2)COOH$	2
4	OCH_3	CH_2COOH	7
5	OCH_3	CH_2CH_2OH	2
6	OCH_3	$CH_2CH(NH_2)COOH$	4

a)The intensity of tryptophan was taken as 100.

The ECL intensities of Trp and other compounds were presented in Tables 1 and 2. The chemiluminescence intensity for indoles, relative to that for Trp, was dependent on the substituent functional groups and their positions. In compounds 1-6 (Table 2) the hydrogen of the indole skeleton of tryptophan was replaced by a functional residue such as hydroxy or methoxy. The intensities of these compounds were weak in comparison with Trp, and indole generated chemiluminescence. However, substituent functions of Trp, such as alanine and other amino acids, did not generate chemiluminescence in acidic solution,$^{1)}$ as was the case for both pyrrol and indoline. In other words, it appears that the indole skeleton is structurally essential for this chemiluminescence reaction. A linear relation was obtained between the amount injected and ECL intensity with in the range of 2-200 pmol. The detection limit was calculated to be 3 times the intensity of the standard deviation of background noise. The detection limit for Trp was about 0.1 pmol and the other indole derivatives were detectable at a level of a few pmol.

In conclusion, a new Trp-Ru(bpy)$_3^{3+}$ ECL system in an aqueous solution has been demonstrated. This FIA using ECR appears to be very promising, and the reaction should prove useful for selective detection of Trp and its related compounds. Furthermore, combination of high-performance liquid chromatography with the FIA for post-column detection is expected to be a specific, simple and sensitive method for determination of compounds present in a wide variety of complex biological samples. This ECL system was potential application for selective and sensitive analysis of Trp.

The author is indebted to Professor A. Sugii for his continuous advice and valuable suggestions, and also to Miss Yuko Banno of this faculty for technical assistance.

References

1) K. Uchikura, Bunseki Kagaku, 39, 323 (1990).
2) K. Kalyanasundaram, Coord. Chem. Rev., 46, 159 (1982).
3) W.K. Nonidez and D.E. Leyden, Anal. Chim. Acta, 96, 401, (1978).
4) D.M. Hercules and F.E. Lytle, J. Am. Chem. Soc., 88, 4745 (1966).
5) I. Rubinstein and A.J. Bard, J. Am. Chem. Soc., 103, 512 (1981).
6) J.B. Noffsinger and N.D. Danielson, Anal. Chem., 59, 865 (1987).
7) W.L. Wallace and A.J. Bard, J. Phys. Chem., 83, 1350 (1979).
8) H.S. White and A.J. Bard, J. Am. Chem. Soc., 104, 6891 (1982).
9) C.R. Bock, J.A. Connor, A.R. Gudemez, R.J. Meyer, D.G. Whitten, B.P. Sullivan, and J.K. Nagle, J. Am. Chem. Soc., 101, 4815 (1979).
10) K. Uchikura, Anal. Sci., 6, 351 (1990).

(Received May 16, 1991)