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Electrochemiluminescent behavior of melatonin and its important derivatives in the presence of $Ru(bpy)_3^{2+}$

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Abstract

Melatonin and some of its important derivatives were found to be able to enhance the ECL of $Ru(bpy)_3^{2+}$ in an alkaline Britton–Robinson buffer solution. The optimum conditions for the enhanced ECL, such as the selection of applied potential mode, type of buffer solution, pH effect and effect of $Ru(bpy)_3^{2+}$ concentration have been investigated in detail in this paper. Under the optimum conditions, the enhanced ECL is linear with the concentration of melatonin and its derivatives over the wide range, and the detection limit for these compounds was found to be in the range of 5.0×10^{-8} to 1.0×10^{-10} mol L^{-1}. The proposed procedure was applied for the determination of drug in tablets with recoveries of 85–93%. A possible mechanism for the enhanced ECL of $Ru(bpy)_3^{2+}$ by melatonin and its derivatives was proposed, and the relationship between molecular structure of melatonin and its derivatives and the enhanced ECL behavior was also discussed.

Keywords: Melatonin and its derivatives; Electrochemiluminescence; $Ru(bpy)_3^{2+}$

1. Introduction

Melatonin (MT) and its important derivatives (MTS), such as 5-methoxyindole-3-acetic acid (MIAA), *N*-acetyl-5-hydroxytryptamine (NAHT), 5-methoxytryptamine (5-MT) and *N*-acetyl-5-methoxy-6-hydroxytryptamine (HMT) (their structures are shown in Table 1) are strongly bioactive, they have regulation action for the genital [1], endocrine [2] and immune [3] system of human. They have recently increased interest as a treatment of sleep disorder and prevention of aging [4]. The contents of these indole-hormones in human body have been used as the important index for clinical examination and diagnosis. Therefore, the development of a method for determination of melatonin and its metabolites or derivatives in biological samples is especially important.

Melatonin in biological samples can be detected by several methods, such as HPLC [5–8], UV [9], fluorimetry [10,11] and RIA [12]. Some chemiluminescent methods have been used for determination of melatonin and its derivatives [13–15]. It has been noted that melatonin and its derivatives belong in the indole aromatic compound. They all have the aromatized conjugation system and can produce the free radical ion under electrolysis [20,22], which is very favorable to give ECL. However, no attention has been paid to use the ECL method for determination of melatonin and its derivatives, even the investigation for ECL of indole compounds has been rarely reported [16–19]. It has been found that melatonin and its derivatives would enhance the ECL of $Ru(bpy)_3^{2+}$ in an alkaline Britton–Robinson (B–R) buffer solution. The aim of the present study is to investigate the behavior of this ECL system and to develop a sensitive ECL method for determination of melatonin and its derivatives. The mechanism of this ECL system has been also investigated

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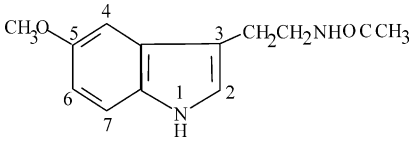
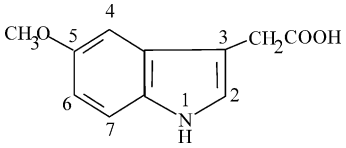
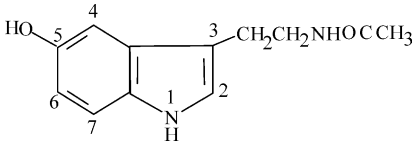
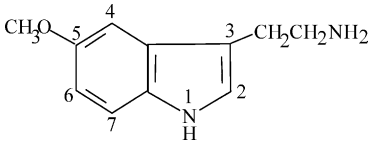
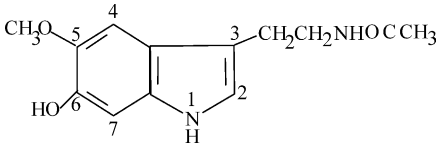
Table 1
Structure of MT and relative derivatives

Compounds	Structure	Abbreviation
Melatonin (*N*-acetyl-5-methoxytryptamine)		MT
5-Methoxyindole-3-acetic acid		MIAA
N-Acetyl-5-hydroxytryptamine		NAHT
5-Methoxytryptamine		5-MT
N-Acetyl-5-methoxy-6-hydroxytryptamine		HMT

and proposed using electrochemical and spectroscopic method.

2. Experimental

2.1. Chemicals and solution

Melatonin (MT), 5-methoxyindole-3-acetic acid (MIAA), *N*-acetyl-5-hydroxy-tryptamine (NAHT), 5-methoxytryptamine (5-MT), *N*-acetyl-5-methoxy-6-hydroxy-tryptamine (HMT) and $Ru(bpy)_3^{2+}$ were purchased from Sigma. Pyridoxine was purchased from Shanghai Chemical Company. Other chemicals were analytical grade or better and double distilled water was used throughout.

The 1.0×10^{-2} mol L^{-1} stock solutions of MTS were prepared by dissolving the required amount of sample in alcohol–water solution (10 + 90, v/v), respectively. 1.0×10^{-2} mol L^{-1} stock solution of $Ru(bpy)_3^{2+}$ was prepared by dissolving the required amount of sample in water. All these stock solutions were stored under refrigeration. Testing solution was prepared by diluting the stock solutions with appropriate buffer solutions before used. The buffer system was a Britton–Robinson (B–R) buffer prepared by titrating a stock solution containing 0.04 mol L^{-1} acetic acid, 0.04 mol L^{-1} phosphoric acid and 0.04 mol L^{-1} boric acid with 0.2 mol L^{-1} sodium hydroxide to the desired pH value.

2.2. Apparatus

2.2.1. ECL detection system

ECL measurements were performed using a system make in our laboratory, consisting of a BPCL Ultra-Weak Chemiluminescence Analyzer (Institute of Biophysics, Academia Sinica, Beijing, China), a potentiostat, an electrochemical cell and a computer control system. A block diagram of the system is shown in Fig. 1. The potentiostat mainly included a waveform generator, which could perform linear-, triangular- and square-wave voltage-sweeps. A conventional three-electrode system was used, and a glassy carbon electrode (GCE) was used as the working electrode, a platinum wire as the counter electrode and an Ag/AgCl electrode (sat. KCl) as the reference electrode. A commercial 5 ml cylindroid glass cell was used as ECL cell, and it was put directly in front of the photomultiplier tube.

2.2.2. Other instruments

A BAS 100A electrochemical analyzer (Bioanalytical Systems, Purdue, IN) was used for all electrochemical measurements.

2.3. Procedure for ECL measurement

One milliliter of sample solution and 1 ml of 1.0 mmol L^{-1} $Ru(bpy)_3^{2+}$ were added to a 10 ml volumetric flask, and