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Full Length Article

Enhanced electrogenerated chemiluminescence of $Ru(bpy)_3^{2+}$/TPrA (bpy=2,2'-bipyridine; TPrA=tri-n-propylamine) via oxygen quenching using melatonin

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ABSTRACT

The effect on the electrogenerated chemiluminescence (ECL) of the $Ru(bpy)_3^{2+}$/TPrA (bpy=2,2'-bipyridine; TPra=tri-n-propylamine) system when melatonin (*N*-acetyl-5-methoxytryptamine; MLT) and structurally related compounds (e.g., D- and L-Tryptophan (TRY), 7-Azatryptophan (7-AZA) and Serotonin (SER)) are present in aqueous buffered solution is reported. MLT, D- and L-TRY, SER and 7-AZA display weak intrinsic ECL when TPrA is used as an oxidative–reductive coreactant. However, micromolar concentrations of melatonin result in up to 2.5-fold enhancement of $Ru(bpy)_3^{2+}$/TPrA ECL while the other analytes attenuate ECL between 2- and 1000-fold. Photoluminescence (PL) emission efficiencies do not change in the presence of melatonin unless the melatonin solution has undergone electrochemical bulk oxidation, at which point PL and ECL are nearly indistinguishable. Spectroscopic, electrochemical and spectroelectrochemical studies indicate that the mechanism involves oxygen scavenging by melatonin oxidation products. This scavenging prevents the quenching of the $^{*}Ru(bpy)_3^{2+}$ excited states by dissolved oxygen in solution. Melatonin can be coupled with 30% by volume 2,2,2-trifluorethanol (TFE) or with the nonionic surfactant Triton X-100 (polyethylene glycol tert-octylphenyl ether) for even greater enhancement of $Ru(bpy)_3^{2+}$/TPrA ECL.

1. Introduction

Electrogenerated Chemiluminescence (ECL) methods provide a powerful set of tools for the study and detection of inorganic, organic and bioactive molecules [1–3]. ECL involves the electrochemical generation of chemical species that react to emit light. For example, in coreactant ECL a chemical species such as tri-n-propylamine (TPrA) and an ECL luminophore such as $Ru(bpy)_3^{2+}$ (bpy=2,2'-bipyridine) are present in the same sample solution [4,5]. Application of a voltage to an electrode results in the oxidation of both coreactant and luminophore. In the case of TPrA a series of following reactions occur that result in the formation of a strong reducing agent (presumably $TPrA^{\bullet}$). This reducing agent can then interact with the oxidized luminophore (i.e., $Ru(bpy)_3^{3+}$) to produce an excited state (i.e., $^{*}Ru(bpy)_3^{2+}$) that emits light [5]. Four mechanisms for $Ru(bpy)_3^{2+}$/TPrA ECL have been proposed [6] that depend on solution pH and the concentration of TPrA, among others. The most commonly invoked mechanism is outlined below:

$$Ru(bpy)_3^{2+} \rightarrow Ru(bpy)_3^{3+} + e^-$$

$$TPrA \rightarrow TPrA^{\bullet +} + e^- \text{ (radical rests on nitrogen atom)}$$

$$TPrA^{\bullet +} \rightarrow TPrA^{\bullet} + H^+ \text{ (radical rests on a carbon atom)}$$

$$Ru(bpy)3^{3+} + TPrA^{\bullet} \rightarrow {}^{*}Ru(bpy)32 + products \rightarrow Ru(bpy)3^{2+} + hv$$

Understanding the ECL properties of melatonin (*N*-acetyl-5-methoxytryptamine, Fig. 1) and structurally similar compounds is of interest for several reasons. Melatonin is a pineal hormone known for its role in numerous physiological processes such as metabolism, reproduction, circadian sleep–wake cycles, appetite, and muscular coordination. It also plays a role in psychiatric processes (such as anxiety and seasonal depression), in the immune system, has anti-oxidant properties [7,8] and is a free radical scavenger [7,9].

Analytical methods for the detection of melatonin and related compounds include voltammetric [10], photoluminescence [11] (PL) and chemiluminescence [12] spectroscopy, liquid [13] and gas [14] chromatography, capillary electrophoresis [15], liquid chromatography coupled with mass spectroscopy [16], and electrogenerated chemiluminescence [17–20]. ECL offers some advantages over other methods of analyses. For example, compared to fluorescent methods those involving ECL do not require an

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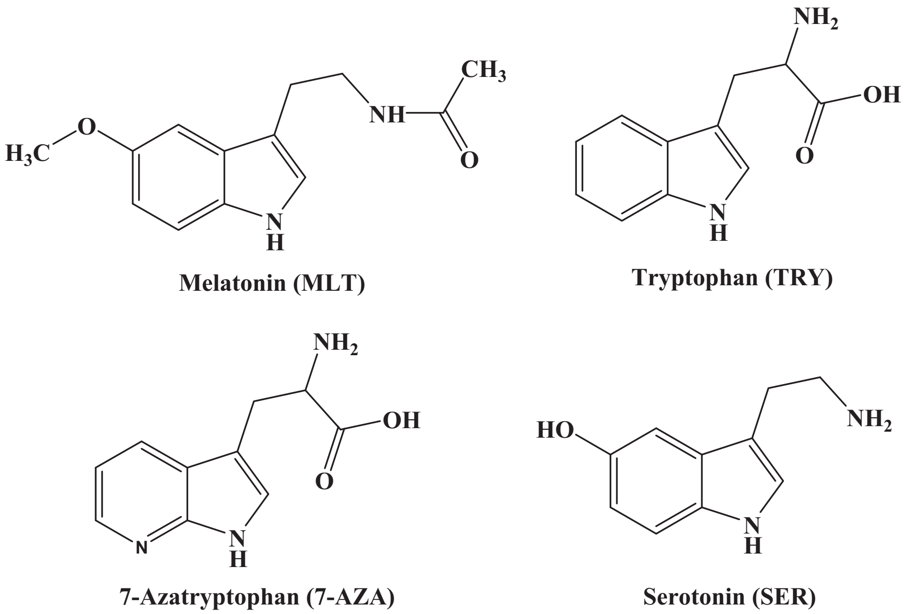

Melatonin (MLT) **Tryptophan (TRY)**

7-Azatryptophan (7-AZA) **Serotonin (SER)**

Fig. 1. Chemical structures of melatonin, tryptophan, 7-azatryptophan and serotonin.

excitation source and tend to be less sensitive to interferences from luminescent impurities and scattered light. Several reports have appeared on the ECL properties of tryptophan [19,20] and serotonin [18] and their quantitative determination in fluid media. In the case of MLT, however, ECL has focused on MLT quenching of the luminol/H_2O_2 system [17]. However, to our knowledge, the ECL of melatonin itself or its effect on the ECL luminophore $Ru(bpy)_3{}^{2+}$ have not been reported.

Therefore, this work focuses on the ECL of melatonin and structurally related compounds (i.e., D- and L-tryptophan, 7-azatryptophan and serotonin, Fig. 1). In particular, a method for the enhancement of the commercially important $Ru(bpy)_3{}^{2+}$/TPrA reaction system via a unique mechanism is presented.

2. Experimental

2.1. Materials

$Ru(bpy)_3Cl_2.6H_2O$ (98%, Strem Chemical, Newburyport, MA), melatonin (Sigma-Aldrich, St. Louis, MO), serotonin (Sigma-Aldrich), 7-azatryptophan (Sigma-Aldrich), tryptophan (Sigma-Aldrich), and tri-*n*-propylamine (98%, Sigma-Aldrich) were used without further purification. Aqueous experiments were performed in either 0.18 M potassium phosphate (Spectrum Chemical Manufacturing Corp., Gardena, CA) buffered solution or Bioveris® assay buffer (approximate composition: 0.18 M TPrA, 0.02 M potassium phosphate buffer, 0.05 M TPrA, 0.1% by weight Triton X-100 at pH = 7.5). Deionized water was purified using a Barnstead E-Pure filtration system.

2.2. Methods

Cyclic voltammetry without photon detection utilized a CH Instruments electrochemical analyzer (Austin, TX) with a glassy carbon working electrode (to prevent adsorption of compound on the electrode surface), a platinum auxiliary electrode and a Ag/AgCl electrode (0.20 V versus the Normal Hydrogen Electrode (NHE)) [21]. The working electrode was cleaned after each run by polishing with 0.05 µm alumina, followed by rinsing with purified and filtered water.

ECL was obtained using two systems. The first, an Origen® Electrochemiluminescence Analyzer (Bioveris Corporation, Gaithersburg, MD) employs a flow injection system that allows rapid and reproducible determinations of sequential samples into a sample chamber. The working and auxiliary electrodes are platinum disks with a Ag/AgCl gel reference electrode downstream from the cell. The detector is a photomultiplier tube positioned directly above the working electrode. In this system, luminophore concentrations were between 10 mM and 0.1 µM in the Bioveris Assay Buffer. The second system couples a conventional three-electrode setup incorporating a CH Instruments electrochemical analyzer and a Hamamatsu HC 135 Photomultiplier Tube (PMT) contained in a "light-tight" box. The Pt working and auxiliary electrodes were cleaned before each run by repeated cycling (+2.0 to −2.0 V) in 6.0 M sulfuric acid followed by sonication for 30 s in dilute nitric acid and rinsing in deionized water and to a Ag/AgCl electrode. Luminophore concentrations in this system were between 1 and 0.01 µM and typically contained millimolar concentrations of analyte (e.g., MLT), 50 mM TPrA, 0.18 M potassium phosphate at pH 8.0 ± 0.2.

UV–vis spectroscopy was performed using a Cary-100 UV–visible Spectrophotometer (Varian Inc., Palo Alto, CA). Photoluminescence spectroscopy and ECL spectra were obtained with a Shimadzu RF-5301 Spectrofluorophotometer (Shimadzu Corporation, Japan). Slit widths for PL spectroscopy were 3 nm and for ECL spectroscopy were 20 nm. Excitation for photoluminescence was at the lowest energy absorption wavelength maximum. Photoluminescence efficiencies (ϕ_{em}; photons emitted per photons absorbed) were obtained relative to $Ru(bpy)_3{}^{2+}$ in water ($\phi_{em}(H_2O) = 0.042$) [22] and ECL efficiencies (ϕ_{ecl} = photons generated per redox event) were obtained by literature methods [23], using $Ru(bpy)_3{}^{2+}$ as the standard ($\phi_{ecl} = 1$):

$$\phi_{ecl} = \frac{\int_0^t I dt}{\int_0^t i dt (N_A/F)}$$

where I is the intensity in photons per second, i is the current in amperes (Coulombs per second), F is Faraday's constant and N_A is Avogadro's constant. Reported values for ECL efficiency are based upon the average of at least three scans with a relative standard deviation of ± 5%.