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Electrochemical behaviour of isatin at a glassy carbon electrode

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Abstract

The electrochemical behavior of isatin – a molecule with a broad range of applications in synthetic, biological and clinical activity – has been investigated over a wide pH range at a glassy carbon electrode (GCE) using cyclic, square wave and differential pulse voltammetry. The oxidation of isatin is an irreversible process, pH dependent and occurs with the formation of a main oxidation product that strongly adsorbs on the electrode surface. The reduction of isatin is also a pH dependent irreversible process. Cyclic voltammograms show two consecutive charge transfer reactions. The diffusion coefficient of isatin was calculated in pH 7.0 phosphate buffer to be $D_0 = 4.9 \times 10^{-7}$ cm^2 s^{-1}. The limit of detection obtained in a solution of pH 7.0 phosphate buffer was LOD = 0.194 μM, based on three times the noise level.

Keywords: Isatin; Indole; Oxidation; Reduction; Voltammetry

1. Introduction

The indole is a powerful pharmacodynamic nucleus and has been reported to possess a wide variety of important biological properties such as anti-inflammatory, antibacterial, anticonvulsant and antioxidant properties [1–3].

Among the indoles, isatin (indole-2,3-dione) (Scheme 1), a versatile heterocyclic compound present in mammalian tissues and body fluids [4,5], is probably one of the most important. The function of isatin as a modulator of different biochemical processes has been a subject of discussion during the past decade. This substance was initially discovered as an inhibitor of monoamine oxidase (MAO), and subsequently identified as a selective inhibitor of MAO B [4,6]. Further investigations have shown that isatin acts as an antagonist of both atrial natriuretic peptide-stimulated [7] and nitric oxide-stimulated [8] guanylate cyclase activity. Isatin has a distinct and discontinuous distribution in brain and other tissues; the highest concentrations (0.1 μg g^{-1} or about 1 μM) in the brain are found in the hippocampus and cerebellum [9,10].

In rat models, stress has been shown to cause an increase of isatin levels in the brain, heart, blood plasma and also increased urinary output of isatin. *In vivo* isatin administration causes a range of dose-dependent behavioral effects, including anxiety at low doses (10–20 mg kg^{-1}) and sedation at higher doses (80–200 mg kg^{-1}) [10–12]. This suggests that different biochemical mechanisms are involved in these diverse effects. However, the functional significance of isatin in the physiology of stress is not yet clearly established.

On the other hand, pathways for the synthesis and metabolism of isatin in animal tissues have not been fully recognized. It has been suggested that isatin is formed in tissues from phenylalanine or tryptophan [7,13]. *In vitro*, isatin can be destroyed easily by a high concentration of hydrogen peroxide. Moreover, isatin is readily metabolized by xanthine oxidase, producing hydrogen peroxide via the formation of superoxide [7,14 and references therein]. However, at this stage it is unclear whether this mechanism operates also *in vivo*.

In recent years, the chemistry of indoles has received a lot of attention due to their wide application for analytical and synthetic purposes. The synthetic versatility of isatin has stemmed from the interest in the biological and pharmacological properties of its derivatives [15]. Although the presence of two carbonyl groups in isatin makes it an attractive target to synthetic organic chemists, little is known about its redox mechanism. Previous studies on the electrochemical reduction behavior of isatin and analog compounds have been undertaken using polarography at mercury electrodes [16–19]. Although

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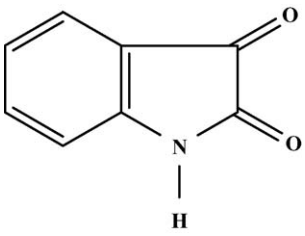

Scheme 1. Chemical structure of isatin.

some investigations concerning the oxidation behavior of isatin have been recently carried out at platinum, gold and glassy carbon electrodes, more work has to be done to fully characterize the isatin redox mechanism.

Investigations of the redox behavior of biologically occurring compounds by means of electrochemical techniques have the potential for providing valuable insights into the biological redox reactions of these molecules. Due to their high sensitivity, voltammetric methods have been successfully used to study the redox behavior of various biological compounds [20–23]. The present study is concerned with probing the redox properties of isatin by using cyclic, square wave and differential pulse voltammetry at a glassy carbon electrode.

2. Experimental

2.1. Materials and reagents

Isatin from Loba-Chemie Indoaustranal Co. (India) was used without further purification. A stock solution of 1 mM isatin was prepared in deionized water and was stored at −4 °C.

All supporting electrolyte solutions (Table 1) were prepared using analytical grade reagents and purified water from a Millipore Milli-Q system (conductivity $\leq 0.1\ \mu S\ cm^{-1}$).

Nitrogen saturated solutions were obtained by bubbling high purity N_2 for a minimum of 10 min in the solution and continuing with a flow of pure gas over the solution during the voltammetric experiments.

Microvolumes were measured using EP-10 and EP-100 Plus Motorized Microliter Pippettes (Rainin Instrument Co. Inc., Woburn, USA). The pH measurements were carried out with a Crison micropH 2001 pH-meter with an Ingold combined glass electrode. All experiments were done at room temperature (25 ± 1 °C).

Table 1
Supporting electrolytes, 0.2 M ionic strength

pH	Composition
1.2	HCl + KCl
2.2	HCl + KCl
3.4	HAcO + NaAcO
4.5	HAcO + NaAcO
5.3	HAcO + NaAcO
6.1	$NaH_2PO_4 + Na_2HPO_4$
7.0	$NaH_2PO_4 + Na_2HPO_4$
8.1	$NaH_2PO_4 + Na_2HPO_4$
9.3	$NaOH + Na_2B_2O_7$
11.1	NaOH + KCl
12.8	NaOH + KCl

2.2. Voltammetric parameters and electrochemical cells

Voltammetric experiments were carried out using a μAutolab running with GPES 4.9 software, Eco-Chemie, Utrecht, The Netherlands. Measurements were carried out using a glassy carbon (GCE) ($d = 1.5$ mm) working electrode, a Pt wire counter electrode, and a Ag/AgCl (3 M KCl) as reference, in a 0.5 mL one-compartment electrochemical cell. The experimental conditions for differential pulse voltammetry (DPV) were: pulse amplitude 50 mV, pulse width 70 ms, scan rate $5\ mV\ s^{-1}$.

The GCE was polished using diamond spray (particle size 1 μm) before each experiment. After polishing, the electrode was rinsed thoroughly with Milli-Q water for 30 s; then it was sonicated for 1 min in an ultrasound bath and again rinsed with water. After this mechanical treatment, the GCE was placed in pH 7.0 0.2 M phosphate buffer electrolyte and various DP voltammograms were recorded until a steady state baseline voltammogram was obtained. This procedure ensured very reproducible experimental results.

2.3. Acquisition and presentation of voltammetric data

All the voltammograms presented were background-subtracted and baseline-corrected using the moving average with a step window of 5 mV included in GPES Version 4.9 software. This mathematical treatment improves the visualization and identification of peaks over the baseline without introducing any artifact, although the peak height is in some cases reduced (<10%) relative to that of the untreated curve. Nevertheless, this mathematical treatment of the original voltammograms was used in the presentation of all experimental voltammograms for a better and clearer identification of the peaks. The values for peak current presented in all graphs were determined from the original untreated voltammograms after subtraction of the baseline.

3. Results and discussion

Initial studies concerning the voltammetric behavior of isatin at a GCE were carried out in pH 7.0 0.2 M phosphate buffer. The cyclic voltammogram in Fig. 1 was obtained in a solution of 330 μM isatin saturated with N_2. During the voltammetric measurement a constant flux of N_2 was kept over the solution surface in order to avoid the diffusion of atmospheric oxygen into the solution of isatin.

Several peaks can be observed in Fig. 1. The reduction and oxidation of isatin occur independently of each other and were investigated separately.

3.1. Oxidation

3.1.1. Cyclic voltammetry

The oxidation of isatin at a GCE was studied by cyclic voltammetry (CV) in pH 7.0 0.2 M phosphate buffer. The CV obtained in a 330 μM isatin solution at a scan rate $\nu = 50\ mV\ s^{-1}$ (Fig. 2)