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Novel electrochemical properties of an emergent mycotoxin: sterigmatocystin

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ABSTRACT

The electrochemical reduction of sterigmatocystin in acetonitrile + 0.1 M tetrabutylammonium perchlorate at glassy carbon electrodes is studied for the first time using cyclic and square wave voltammetries, and controlled-potential electrolysis. Two reduction peaks centered at −1.77 and −2.33 V vs. Ag/AgCl were found, which correspond to the formation of the radical anion and dianion, respectively. The overall electrode process was diffusion controlled. The initially formed radical anion undergoes an irreversible dimerization reaction with a rate constant of $2.06 \times 10^4\ M^{-1}\ s^{-1}$, producing a basic dimeric dianion, which is protonated by the starting molecule. An unstable dimeric reaction product was detected by UHPLC-MS/MS measurements. A value of $(3.1 \pm 0.9) \times 10^{-5}\ cm^2\ s^{-1}$ was calculated for sterigmatocystin diffusion coefficient from convoluted cyclic voltammograms. Thermodynamic and kinetics parameters were determined from digital simulation of cyclic voltammograms. Probable chemical structures for the dimer are proposed based on the results of theoretical calculations. The effect of the addition of tetrabutylammonium hydroxide, trifluoroacetic acid and water on the voltammetric signals was also investigated. The quantitative determination of sterigmatocystin was carried out by square wave voltammetry using the commercial reagent. The calibration curve was linear in the concentration range from 0.050 to 11.2 ppm, and the limits of detection and quantification were 10 and 33 ppb for signal to noise ratios of 3:1 and 10:1, respectively.

1. Introduction

Mycotoxins are toxic secondary metabolites produced by different kinds of fungi [1]. Ingestion, inhalation and/or dermal adsorption of mycotoxins can produce illness or death of animal and human being [2].

Sterigmatocystin (STEH) was first isolated in 1954 from *Aspergillus versicolor* cultures [1]. However, it has been reported that STEH can be also produced by fungal species phylogenetically and phenotypically different such as *Aschersonia*, *Aspergillus*, *Bipolaris*, *Botryotrichum*, *Chaetomium*, *Emericellai*, *Eurotium*, *Farrowia*, *Fusarium*, *Humicola*, *Moelleriella*, *Monocillium* and *Podospora* [3].

STEH is a precursor of aflatoxin B1 in the biological transformation [4]. Chemical structures of STEH and aflatoxin B1 are similar (Fig. 1). Acute toxicity, carcinogenicity and the metabolism of STEH are compared to aflatoxin B1 and other hepatotoxic mycotoxins. Thirty-three species of *Aspergillus* may produce STEH [3]. There are more species that produce STEH (55 spp) than aflatoxins (13 spp).

The International Agency for Research on Cancer (IARC) classifies STEH in the group 2B (possible human carcinogen) [5] due to its toxicological, mutagenic and carcinogenic effects in animals [6].

Although aflatoxins are considered 150–200 times more powerful than STEH, the quantities of STEH produced by some strains under optimal conditions minimize this difference [7]. Studies on STEH are mainly focused on the mechanism of toxin producing, toxin contamination and carcinogenic effects [8–10].

No country has legislation related to STEH levels permitted in food. However, some countries set STEH maximum levels allowed in some food. Thus, Czech Republic and Slovakia allow a level of 50 ppb for rice, vegetables, potatoes, flour, chicken, meat and milk, and of 20 ppb for other foods. The Department of Health of California (USA) proposes a LD_{50} of 8 μg/kg of body weight for an adult of 70 kg [11], being LD_{50} the amount of a toxic substance that kills 50% of a group of test animals [1].

Relatively high levels of STEH (in the order of ppm) were detected in housing and construction materials contaminated with *A. versicolor* [12]. The post harvest contamination of cereals with *Aspergillus* fungi involves risk to human health due to the potential production of STEH and other mycotoxins such as aflatoxin B1 and ochratoxin A [13].

STEH was found in 55 of 215 samples of different grains (barley, wheat, buckwheat, rye) in Latvia in a concentration range between 0.7 and 83 ppm [14]. There is only one report related to the presence of STEH in beer. STEH was found in 2 of 26 samples analyzed in the concentration range from 4 to 7.8 ppm [15].

A. versicolor is often present in cheese, whereas aflatoxin producing fungi as *A. flavus* and *A. parasiticus* are rarely present in this food.

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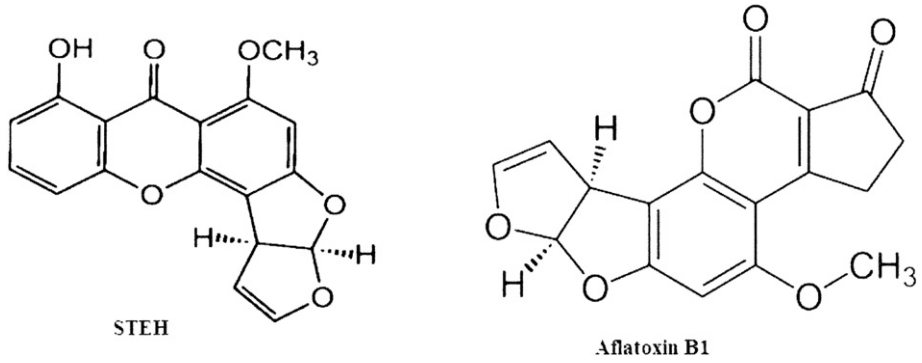

Fig. 1. Chemical structures of sterigmatocystin and aflatoxin B1.

Thus, the presence of STEH was detected in different kinds of cheese [16–19]. However, aged cheese (over six months) inhibits the production of STEH [20].

So far, the most common analytical methods used for the determination of STEH in food are: ELISA immunoassays [21–23], chromatographic methods [6] and enzyme biosensors [24,25]. However, chromatographic methods are the most widely used.

In this study, we discuss the electrochemical reduction of STEH at glassy carbon (GC) electrodes in acetonitrile (ACN) + 0.1 M tetrabutylammonium perchlorate (TBAP) using cyclic (CV) and square wave (SWV) voltammetries, and controlled potential electrolysis. We evaluated three alternative reaction mechanisms; however, one of them is the most likely based on the results of digital simulation of cyclic voltammograms and results of controlled potential electrolysis. Moreover, we also propose the most probable structures of the reaction products based on theoretical calculations. SWV was used to perform the quantitative determination of STEH using the commercial reagent.

2. Experimental

2.1. Reagents

STEH was purchased from Santa Cruz Biotechnology, USA, and used as received. ACN was Sintorgan (HPLC degree). It was first distilled over P_2O_5 (Fluka) and the distillate was dried over 3 Å molecular sieves during 48 h prior to use. TBAP (Fluka, electrochemical degree) was dried at 25 °C in a vacuum oven during 24 h and then the temperature was gradually raised to 60 °C and maintained during 24 h. Finally, it was stored in a desiccator.

Tetrabutylammonium hydroxide (TBAOH, 1.0 M in methanol) was Fluka, trifluoroacetic acid (TFA) was Sigma-Aldrich and acetone (Ac) was Sintorgan (HPLC degree). They were used as received. TBAOH solution was titrated previously to the experiments. $NaClO_4$ (Merck p.a.) was used as supporting electrolyte in controlled potential electrolysis measurements at a concentration of 0.1 M. It was first dried in a vacuum oven at room temperature and then the temperature was gradually increased to 180 °C. Then, it was stored in a desiccator.

STEH stock solutions (1.5×10^{-2} M) were prepared in ACN and kept in the refrigerator. Working solutions were prepared daily by adding aliquots of the stock solution to ACN + 0.1 M TBAP. The STEH bulk concentration (c^*_{STEH}) was varied from 3.1×10^{-8} to 5.1×10^{-3} M for voltammetric measurements. A $c^*_{STEH} = 5.6 \times 10^{-4}$ M was used for performing controlled potential electrolysis measurements. In all cases, final concentrations were calculated through UV–vis absorption measurements.

For security reasons and considering the toxicity of STEH, all solutions and experimental measurements were carried out using latex gloves and a facial mask. The residues were discarded in special bottles properly labeled. They were periodically collected by a company responsible for their final destination.

2.2. Apparatus and experimental measurements

Voltammetric and controlled potential electrolysis measurements were performed with an AutoLab PGSTAT 12 potentiostat, controlled by GPES 4.9 electrochemical software from EcoChemie, The Netherlands. In CV, the scan rate, v, was varied from 0.025 to 4 V s^{-1}. The characteristic parameters in SWV were: amplitude, ΔE_{SW} = 50 mV, staircase height, ΔE_s = 10 mV, and the frequency, f, was varied from 10 to 200 Hz.

A two-compartment Pyrex cell with a volume of 2 mL was used to perform voltammetric measurements [26]. The working electrode was a GC disk (BAS, 3 mm diameter). It was polished using 0.3 and 0.05 μm wet alumina powder (from Fischer), copiously rinsed with H_2O and sonicated in a water bath during 2 min. Finally, it was rinsed with Ac and dried under an air flow. Its electrochemical area, A, was determined through amperometric measurements [27] using a solution of 9.87×10^{-4} M $K_4[Fe(CN)_6]$ + 0.50 M KNO_3 at 25 °C. A value of 7.6×10^{-6} cm^2 s^{-1} was obtained from the literature for the $K_4[Fe(CN)_6]$ diffusion coefficient [28]. An average value of A = (0.08 ± 0.02) cm^2 was determined from four replicated measurements. The counter electrode was a large-area platinum foil (A ~ 2 cm^2). The reference electrode was Ag/AgCl (3 M NaCl). The cell for controlled potential electrolysis was one of the three-compartment types [29]. A fiberglass paper separated the working and counter electrode compartments. The working electrode was a rotating GC rod with a geometric area of 3.52 cm^2, the pseudo-reference electrode was a Ag wire and the counter electrode was a Pt foil of large area [29]. The oxygen concentration was minimized by bubbling pure argon saturated with the blank solution through the STEH solution for about 20–25 min, until the oxygen classical reduction peak at about −1.0 V disappeared [30]. Then, an argon atmosphere was kept above the solution in the cell throughout the experiment.

The positive-feedback technique was used in all experiments to compensate the solution resistance. The temperature was 25 °C.

Cyclic voltammograms were convoluted, after subtraction of background currents, by applying the method proposed by Oldham [31]. The fitting of experimental cyclic voltammograms was performed using BAS DigiSim®.

UV–vis absorption spectra were recorded using a Hewlett Packard model 8452A spectrophotometer equipped with temperature controller. Silica cells were 1 cm path length. STEH has two absorption maxima at λ = 240 nm and λ = 321 nm in ACN. In addition, an absorption peak of low absorptive appears at 280 nm. Absorption spectra recorded at different c^*_{STEH} showed that STEH satisfies to the Lambert and Beer Law. Molar extinction coefficients, ε, were ε_{240} = (3.72 ± 0.01) × 10^4 M^{-1} cm^{-1} and ε_{321} = (1.62 ± 0.01) × 10^4 M^{-1} cm^{-1}.

The UHPLC-MS/MS system consisted of an Acquity™ Ultra High Performance LC system (Waters, Milford) equipped with auto-sampler injection and pump systems (Waters, Milford). The auto-sampler vial tray was maintained at 15 °C. It was coupled to a Mass Spectrometry analyzer with a Quattro Premier™ XE Micromass MS Technologies triple