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Research paper

Carbon nanotube-based electrochemical biosensors for determination of *Candida albicans*'s quorum sensing molecule

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ABSTRACT

Candida albicans produces various quorum sensing molecules (QSMs) which are involved in biological processes, e.g. morphological changes or biofilm formation. Thus, a sensitive and selective electrochemical assay was developed for determination of tryptophol as one of the most important QSMs. Preparation, characterization, and testing the electrocatalytic activity of prepared sensors with various sensing elements and nanomaterials were investigated. In addition, the effects of supporting electrolytes, pH of buffer solutions, scan rate, and possible interferences on the response of tryptophol's sensor were studied. Eventually, improving the sensitivity and selectivity were achieved using a combination of 12-crwon-4-ether and MWCNTs (10% w/w for each). With the developed electrochemical sensor, the linear response was observed from 10 μg/ml to 110 μg/ml with the detection limit of 1 μg/ml and regression coefficient of 0.998. Consequently, the method was successfully applied for the detection of tryptophol in the fungal culture.

1. Introduction

Immuno-suppressed patients are highly susceptible to infections by opportunistic pathogens such as *Candida* species [1,2]. Therefore, opportunistic human fungal pathogens have become increasingly important over the last decades [3,4]. *Candida albicans* can colonize skin and mucosal surfaces of healthy people and thus occurs commensally in the gastrointestinal tract, oral cavity and vagina [5]. Moreover, *C. albicans* can enter the bloodstream by direct penetration from the epithelium after tissue damage, or by dissemination from biofilms formed on medical devices implemented into the patient's organs, e.g. catheters, dental implants, endoprostheses or artificial joints [6]. Thus, the interactions between this organism and the host tissues are highly complex, regulated by several factors such as secretion of quorum sensing molecules [7].

In this regards, *C. albicans* produces various aromatic alcohols, farnesol [8], tyrosol [9,10], phenylethanol [11], and tryptophol [12,13], which have quorum sensing properties or are involved in biological processes, e.g. morphological changes or biofilm formation [14–17].

From the diagnostic point of view, the use of classical methods for the detection and analysis of microbial diseases, such as biological (culturing), microscopic evaluations or complex analytical protocols, are expensive, invasive or require time-consuming procedures [7]. Due to the low sensitivity of the classical protocols, a new sensitive detection method is still needed.

Microbial electrochemical systems have been suggested as promising alternative tools for microbial detection [18–20]. They can be effectively used for the distinguishing between the dead and leaving organisms. Since those systems rely on the interactions of the whole microbial cells with the electrode surface, low sensitivity and simplicity still exist.

Recently, extracellular measurements of metabolites secretion redirected the research attention towards a better detection assays [21].

Therefore, focusing on the electrochemical determination of secreted quorum sensing molecules in the microbial culture of *C. albicans* is the main concern of the current study. In this regards, tryptophol was assigned as the potential candidate. The metabolic conversion of tryptophan, as an essential amino acid, to tryptophol (a secondary product of alcoholic fermentation) was first described by Felix Ehrlich in 1912 [22]. As has been reported in the literature, spectrophotometric, mass spectrometry and HPLC methods

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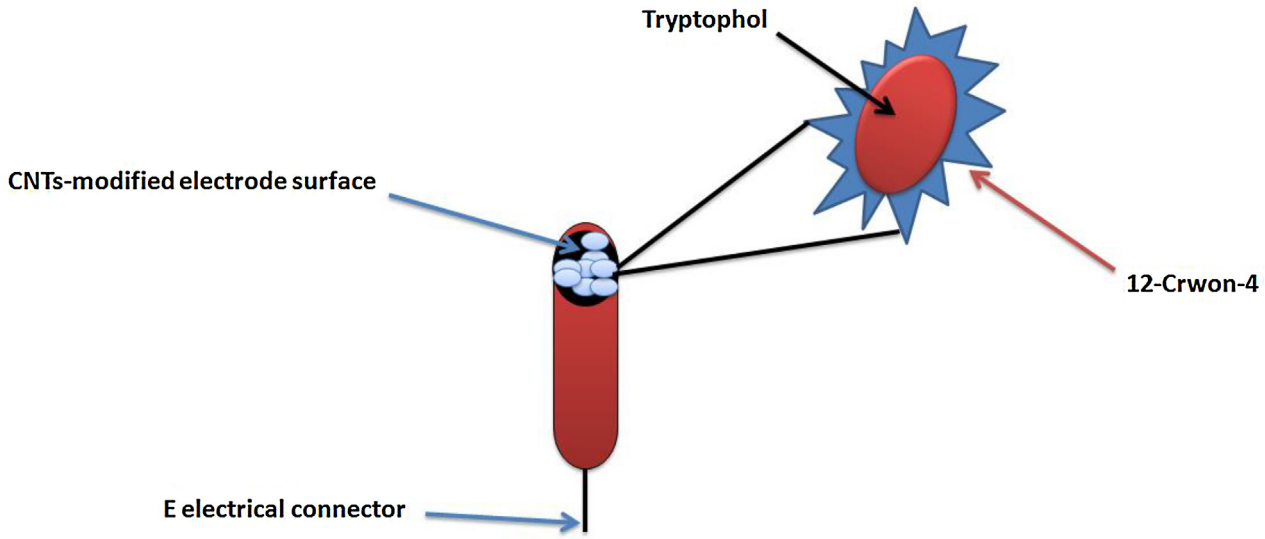

Scheme 1. Schematic representation of the functionalized working electrode surface with 12-crown-4 and MWCNTs (10% each) to selectively interact with the tryptophol as a quorum sensing molecule.

were developed for the determination of tryptophol, tyrosol and phenylethanol in the microbial culture [23–25].

From the electroanalytical point of view, no electrochemical studies were performed on any of the quorum sensing molecules of *C. albicans*. Thus, studying the bioelectrochemistry of tryphtophol and determination of its amount in the fungal culture is the objective of this study. Sensor modifications with macromolecules as sensing agents and carbon nanotubes will be used as essential electrode compositions for the selectivity, sensitivity and electrocatalytic activity purposes. Preparation, characterization and testing the activity of the modified electrodes will be conducted to reach the optimum assay conditions (Scheme 1).

2. Materials and methods

2.1. Chemicals and apparatuses

All reagents were of the analytical grade and bidistilled water was used throughout the experiments. Tryptophol and 2-(4-Hydroxyphenyl) ethanol (tyrosol) were purchased from Aldrich. Yeast Nitrogen Base (YNB) without amino acids, tryptophan, were purchased from Sigma. Different cyclic macromolecules were tested as sensing ionophores including; native α-, β- and γ-cyclodextrin (Sigma), 12-crown-4 ether (Fluka), 15-crown-5 ether (Fluka), 21-crown-7 ether (Fluka), 24-crown-8 ether (Fluka), 30-crown-10 ether (Fluka), calix[4]arene and calix[8]arene (Aldrich). Paraffin oil (Fluka), and synthetic carbon powder 1–2 μm (Aldrich) were used for preparation of the carbon paste electrode. Different carbon nanomaterials including: multiwall carbon nanotubes (MWCNTs, Aldrich, CAS Number 308068-56-6 for MWCNTs), single wall carbon nanotubes (SWCNTs, Aldrich, CAS Number: 308068-56-6) were used.

All electrochemical measurements were performed using a computer controlled Gamry Potentiostat/Galvanostat/ZRA G750, connected to a three electrode system comprising a CNTs-CPE paste working electrode, a Pt disc auxiliary electrode and Ag/AgCl/3 M KCl reference electrode. The pH measurements were performed using Metrohm-pH-meter.

2.2. Sensor construction and electrochemical procedures

The Crown ether/MWCNTs modified carbon paste electrodes were prepared by thoroughly hand-mixing 75 mg of MWCNTs, 75 mg of 12-Crown-4, and 600 mg of synthetic graphite powder with 200 μl of paraffin oil in a small mortar. The prepared homogeneous-paste was packed into the electrode assembly with a surface area of 3 mm, **and a height of 20 mm.** Electrode surface regeneration was performed by polishing with a wet filter paper till a shiny and very smooth electrode surface was obtained.

Prior to each electrochemical measurement, the prepared sensor was electrochemically activated in Britton–Robinson buffer (BR buffer, pH 7) by 10-cyclic scans from −0.2 to 1 V with scan rates of 50 mV/s. Cyclic voltammetry (CV) and Linear Sweep Voltammetry (LSV) were used for sensors characterizations, assay optimizations, whereas the signals were recorded in the potential range from 0.2 to 1.0 V with scan rates of 50 mV/s without stirring at room temperature.

2.3. Determination of tryptophol in Candida albicans culture

C. albicans was cultivated overnight in 250 ml flasks with 50 ml of a complex medium (Yeast Peptone Dextrose (YPD)) at 30 °C. A pre-culture was prepared by diluting the overnight culture to an optical density (OD_{620}) of 0.2 in 25 ml YPD. The cells were harvested by centrifugation (Eppendorf centrifuge 5804R) at 5000 rpm at room temperature for 5 min and washed with phosphate buffer pH 7 [7,18]. Consequently, four working cultures were prepared from defined medium (Yeast Nitrogen Base (YNB)) without amino acids supplemented with 2% glucose and 0 mg/l, 20 mg/l, or 100 mg/l of tryptophan.

For measuring the bioelectrochemical signals, after 3 h of incubation with different tryptophan concentrations, aliquots of the metabolically active cell suspension were introduced into the electrochemical cell, and the cyclic voltammograms (CV) were recorded in the potential range from 0.2 to 1 V, with scan rate of 50 mV/s, without stirring at room temperature. All potentials are referred to Ag/AgCl/3 M KCl reference electrode.