Biosensors and Bioelectronics 49 (2013) 192–198

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Direct electrochemical determination of *Candida albicans* activity

Rabeay Y.A. Hassan [a,b,*], Ursula Bilitewski [a]

[a] Helmholtz Centre for Infection Research, Working Group BiSA, Inhoffenstr. 7, 38124 Braunschweig, Germany
[b] Microanalysis Lab, Applied Organic Chemistry Department, National Research Centre (NRC), Eltahrir Street, 12311-Dokki, Cairo, Egypt

ARTICLE INFO

Article history:
Received 25 February 2013
Received in revised form
24 April 2013
Accepted 7 May 2013
Available online 15 May 2013

Keywords:
Bio-electrochemistry of *Candida albicans*
Pathogen detection
Mitochondrial respiratory chain
Cytochrome c oxidase

ABSTRACT

Despite advances made in the field, rapid detection methods for the human pathogen *Candida albicans* are still missing. In this regard, bio-electrochemical systems including electrochemical sensors and biosensors satisfy the increasing demand for rapid, reliable, and direct microbial analyses. In this study, the bioelectrochemical characteristics of *C. albicans* were investigated for use in an analytical system that determines the viability of the organisms. The electrochemical responses of viable and non-viable cells of *C. albicans* and *Saccharomyces cerevisiae* were monitored. Cyclic voltammograms (CV) showed an irreversible oxidation peak at about 750 mV that accounts for viable cells. The peak current increased at viable cell numbers ranging from 3×10^5 to 1.6×10^7 cells/ml, indicating that the amount of viable cells can be accurately quantified. To elucidate the underlying electron transfer processes, the influence of electron transfer chain (ETC) – inhibitors on the electrochemical behavior of the two organisms were investigated. Inhibition of the function of classical respiratory chain (CRC) led to a decrease in the electrochemical response, whereas the oxidation current increased when the alternative oxidase (AOX) pathway was blocked by salicylhydroxamic acid (SHA). Blocking the AOX pathway improved the electrochemical performance, suggesting an involvement in the CRC, with cytochrome c oxidase (COX) as a relevant protein complex. Mutants, in which components of COX were deleted, showed a lower electro-activity than the wild-type strain. Particularly, deletion of subunit *COX5a* almost completely abolished the electrochemical signal. We believe that this work can be utilized for the development of early detection assays and opens the door for new technological developments in the field of *C. albicans*.

1. Introduction

Candida albicans is a polymorphic fungus that causes a broad spectrum of diseases in humans, ranging from localized mucosal infections to systemic candidiasis. The most important virulence factor of *C. albicans* is the morphological switch between yeast and filamentous forms (Mayer et al., 2013; Pitarch et al., 2006). Since it is an opportunistic pathogen, mainly patients with a compromised immune system are at risk to develop systemic candidiasis. As detection of microorganisms usually requires several days, in intensive care units of hospitals antimycotics are frequently given prophylactically to prevent *C. albicans* infection. Yet, this could be prevented with simple and rapid diagnostic tools.

The classical detection methods of viable microorganisms rely on the cultivation and growth of the organisms on agar plates or in liquid culture. Subsequently, colony forming units (CFUs) or cell numbers, the dry cell weight or the turbidity of samples is assessed (Keer and Birch, 2003).

PCR technique is extremely sensitive and may also allow further characterization of the pathogen (Gilbride et al., 2006; Bekal et al., 2003). However, both molecular methods cannot distinguish between viable and non-viable organisms, so that the infectious risk may be overestimated (Girones et al., 2010). Measuring the efficiency of microbial respiration and the activity of the electron transport chain (ETC) are considered to be suitable indicators of cellular activity, because they are essential for the replication of aerobic organisms. In eukaryotes, the respiratory chain is located in mitochondria where more than 90% of the total oxygen is consumed via the ETC. Therefore, the oxygen uptake rate of a microbial culture is a direct indicator of the respiratory activity (Wesolowski et al., 2008). Moreover, redox compounds, such as tetrazolium salts (Tsukatani et al., 2009, 2003, 2008), resazurin (Alamar blue) (Byth et al., 2001; Mendoza-Aguilar et al., 2012; Watanabe et al., 2009), and various quinoid compounds, can be used in colorimetric assays to assess viability and/or proliferation of living cells. However, these assays are not suitable for routine laboratory use due to the long incubation time of the redox mediators in the microbial culture and interference with the optical density measurement.

Alternatively, electron transfer processes from microorganisms towards electrodes in bio-electrochemical systems can be exploited for the development of microbial fuel cells (Schaetzle O and Baronian, 2008) or diagnostic tools (Lovley, 2012) that provide a rapid

* Corresponding author at: Microanalysis Lab, Applied Organic Chemistry Department, National Research Centre (NRC), Eltahrir Street, 12311-Dokki, Cairo, Egypt.
E-mail address: rabeayy@yhaoo.com (R.Y.A. Hassan).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.05.015

assessment of microbial activity (Junil et al., 2012; Marsili et al., 2008b). In bio-electrochemical systems, either the direct interaction between microorganisms and electrodes can be used, or the microbe–electrode communication can be induced with natural (Wang et al., 2007) or artificial redox mediators (Gottschamel et al., 2009; Heiskanen A et al., 2004; Roller SD et al., 1984). Electrochemical detection methods that are based on redox mediators are applicable when the reduced electron acceptor can be oxidized at electrodes (Congdon et al., 2013; Junil et al., 2012). Since these compounds have to be added to the sample. The most simplistic approach is a mediator-less electrochemical system, which relies on the transfer of electrons from an electrochemical active microorganism to the electrode surface (Bullen et al., 2006). Mediator-less system for the detection of microorganisms are known since the late 1970s (Lovley, 2012; Matsunaga et al., 1979; Matsunaga and Namba, 1984).

In this study, we have focused on the bio-electrochemistry of *C. albicans* (target organism). *C. albicans* offers the opportunity to explore factors that play a key role in the interaction of the microorganism with electrodes, as the structure of its respiratory chain is significantly different from that of *Saccharomyces cerevisiae* (model organism) (Joseph-Horne et al., 2001a). *C. albicans* has three respiratory pathways (Fig. 1): The classical respiratory chain (CRC), an alternative oxidative pathway (AOX) (Huh and Kang, 2001; Joseph-Horne et al., 2001b; Veiga et al., 2003) and a parallel electron transport chain (PAR) (Ruy et al., 2006). The CRC comprises all four enzymatic complexes including the proton pumping complex NADH dehydrogenase (complex I), which is missing in *S. cerevisiae*. The AOX catalyzes the direct oxidation of ubiquinol by oxygen and, thus bypass complexes III and IV which enable respiration even in the presence of downstream CRC inhibitors. The PAR is only activated when both the CRC and the AOX pathways are totally blocked, allowing electron flux to be redirected upstream of complex III, parallel to the CRC (Ruy et al., 2006).

In our previous study, we have developed a mediated bioelectrochemical system for the detection of *C. albicans*'s viability in which electrons are shuttled from the NADH-dehydrogenases of ETC (Hassan and Bilitewski, 2011). Recently the early detection of *C. albicans* biofilms at porous electrodes has been reported (Congdon et al., 2013). Intriguingly, the direct electron transfer capability of *C. albicans*, which provides a better mean to determine the biological function of respiratory pathways of *C. albicans*, has not been reported yet.

Therefore, we investigated the direct bio-electrochemistry of the target organism as well as the involvement of the respiratory pathways in the electrochemical signal. Voltammograms from suspensions of *C. albicans* and *S. cerevisiae* showed an irreversible oxidation peak at around 750 mV, indicating that electrons were transferred from the cells to the electrode surface. Since non-viable cells do not exhibit any electrochemical activity, the generated oxidation current reflects the cell viability status. The peak height correlated with the cell numbers and increased with the cultivation time. To obtain a more detailed understanding of the relevant electron transfer step, ETC-inhibitors were used. The peak current decreased when the CRC pathway was inhibited. In addition, the electrochemical activities of COX mutants of *S. cerevisiae* were significantly lower than those of the wild type. In particular, the deletion of subunit *COX5a* almost completely abolished the electrochemical response. This confirms the essential role of COX (complex IV) in the interaction between yeasts and electrodes.

2. Materials and methods

2.1. Materials and instruments

Potassium phthalate mono basic was purchased from Riedel-de Haen, and a 100 mM solution was adjusted to pH 7 by KOH. YPD broth, antimycin A from *Streptomyces sp.*, and rotenone were purchased from Sigma. YPgal medium contained yeast nitrogen base with amino acids from Sigma, peptone from Roth, and galactose from Merck. Salicylhydroxamic acid (SHA) was from Aldrich. Synthetic carbon powder was obtained from Sigma-Aldrich and paraffin oil from Fluka.

All OD measurements were performed in 180 µl sample volumes with the microtiter plate reader µQuant (BioTek Instruments GmbH, Bad Friedrichshall, Germany), and fluorescence was determined with the microtiter plate reader Cytofluor, Reader Series 4000 (PerSeptive Biosystems, Framingham, USA). The electrochemical measurements were carried out with a computer-controlled Gamry Potentiostat/Galvanostat/ ZRA G750 (Gamry, Pennsylvania, USA), which was connected to a three-electrode electrochemical cell with a carbon paste working electrode, a Pt disc auxiliary electrode, and a KCl saturated Ag/AgCl reference electrode.

2.2. Preparation of microbial samples

2.2.1. Standard cultivation conditions

The following fungal strains were used: *C. albicans* strains CAF2-1 and *S. cerevisiae* BY4741 and its single gene deletion mutants *COX5a*, *COX5b*, *COX7*, *COX8*, and *COX12* (Euroscarf,

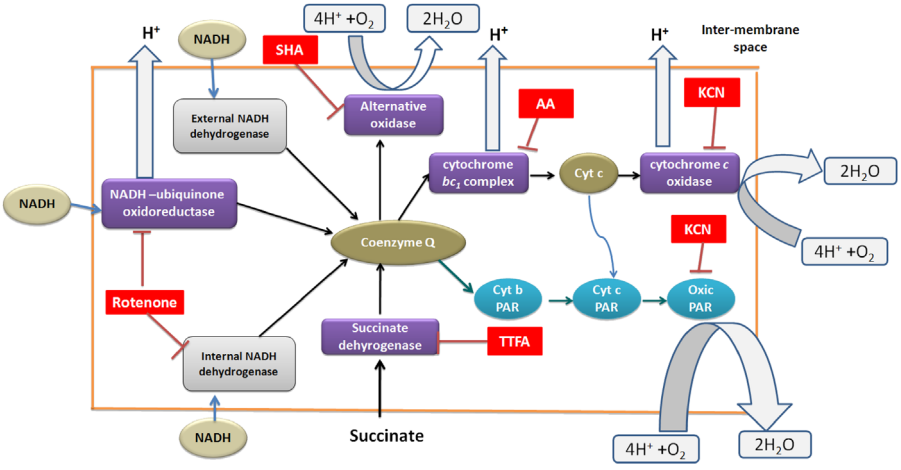

Fig. 1. Respiratory chain structure of *C. albicans*. NADH is oxidized by NADH-dehydrogenases leading to reduced coenzyme Q. This is also produced from the oxidation of succinate by succinate-dehydrogenase. Reduced coenzyme Q is oxidized again either by oxygen through the alternative oxidase, or by cytochrome c in the cytochrome bc_1 complex. Cytochrome *c* is finally oxidized by cytochrome *c* oxidase. These latter reactions can also be catalyzed by components of the parallel pathway PAR.