Biosensors and Bioelectronics 87 (2017) 654–668

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Recent advances in cytochrome *c* biosensing technologies

Pandiaraj Manickam [a,*], Ajeet Kaushik [b], Chandran Karunakaran [c], Shekhar Bhansali [a]

[a] Bio-MEMS and Microsystems Laboratory, Department of Electrical and Computer Engineering, Florida International University, Miami, FL, USA
[b] Center for Personalized Nanomedicine, Institute of Neuro immune Pharmacology, Department of Immunology, Herbert Wertheim College of Medicine, Florida International University, Miami, FL, USA
[c] Biomedical Research Laboratory, Department of Chemistry, VHNSN College (Autonomous), Virudhunagar, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form
31 August 2016
Accepted 2 September 2016
Available online 4 September 2016

Keywords:
Cytochrome *c*
Point-of-care
Apoptosis
Cancer therapy
Biosensors
Enzymes
Antibodies
Aptamers

ABSTRACT

This review is an attempt, for the first time, to describe advancements in sensing technology for cytochrome *c* (cyt *c*) detection, at point-of-care (POC) application. Cyt *c*, a heme containing metalloprotein is located in the intermembrane space of mitochondria and released into bloodstream during pathological conditions. The release of cyt *c* from mitochondria is a key initiative step in the activation of cell death pathways. Circulating cyt *c* levels represents a novel in-vivo marker of mitochondrial injury after resuscitation from heart failure and chemotherapy. Thus, cyt *c* detection is not only serving as an apoptosis biomarker, but also is of great importance to understand certain diseases at cellular level. Various existing techniques such as enzyme-linked immunosorbent assays (ELISA), Western blot, high performance liquid chromatography (HPLC), spectrophotometry and flow cytometry have been used to estimate cyt *c*. However, the implementation of these techniques at POC application is limited due to longer analysis time, expensive instruments and expertise needed for operation. To overcome these challenges, significant efforts are being made to develop electrochemical biosensing technologies for fast, accurate, selective, and sensitive detection of cyt *c*. Presented review describes the cutting edge technologies available in the laboratories to detect cyt *c*. The recent advancements in designing and development of electrochemical cyt *c* biosensors for the quantification of cyt *c* are also discussed. This review also highlights the POC cyt *c* biosensors developed recently, that would prove of interest to biologist and therapist to get real time informatics needed to evaluate death process, diseases progression, therapeutics and processes related with mitochondrial injury.

Contents

* Corresponding author.
E-mail address: pmanicka@fiu.edu (P. Manickam).

http://dx.doi.org/10.1016/j.bios.2016.09.013
0956-5663/

1. Introduction

1.1. Cytochrome c, a brief understanding

Cytochrome *c* (cyt *c*), a small (molecular weight: ~ 12,500 Da, 104 amino acids), highly water-soluble redox active metalloprotein is located in the intermembrane space of mitochondria (Hayashi and Capaldi, 1972; Martinou et al., 2000). It has a highly conserved three dimensional (3D) structure and a covalently attached active heme prosthetic group (Fig. 1). In mitochondrial respiratory chain of energy production, cyt *c* functions as a single electron carrier between two membrane bound complexes viz. complex III and (bc_1 complex or cytochrome *c* reductase) and complex IV (cytochrome *c* oxidase). During the mitochondrial electron transfer reaction, the heme active site of cyt *c* alternates between a reduced ferrous (Fe^{2+}) and oxidized ferric (Fe^{3+}) states (Mathews, 1985; Skulachev, 1998; Wang et al., 2002). Cyt *c* is a multi-functional enzyme, involving in both life and death decisions of cell. It participates in electron transfer as a part of the mitochondrial electron transport chain (ETC) and is thus an indispensable part of the energy production process. Its release from mitochondria is an essential step for the formation of the apoptosome and the progression of cell death processes (Hüttemann et al., 2011; Wang, 2001).

1.2. Role of cyt c in mitochondrial electron transport chain

Mitochondria are often referred to as “powerhouse of the cell”, because they produce approximately 90% of the required energy in the form of adenosine triphosphate (ATP) through oxidative phosphorylation. Thus, the primary role of oxidative phosphorylation is the production of energy, which drives all cellular processes.

Mitochondrial ATP production occurs through the flow of electrons from nicotinamide adenine dinucleotide (NAD) or flavin adenine dinucleotide (FAD) reducing equivalents. These electrons are passed through a series of respiratory complexes in the inner mitochondrial membrane called electron transport system (Fig. 2). It results in the formation of an electrochemical gradient, which enables the ATP synthase to synthesize the energy rich ATP. The mitochondrial respiratory chain comprises series of five multi-subunit enzyme complexes (complexes I, II, III, IV and V) and two electron carriers (ubiquinone and cyt *c*). These two electron carriers play critical role in the efficient transfer of electrons in the electron transport chain (Hatefi, 1985; Koopman et al., 2007; Kushnareva et al., 2002).

1.3. Role of cyt c in apoptosis

In addition to its central role as power source, mitochondrial respiratory chain is also the major source for generation of reactive oxygen species (ROS) (Kannan, 2000). Under normal physiological conditions, 1–2% of molecular oxygen consumed by mammalian cells is metabolized to ROS. Consequently, the ROS including superoxide anion radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH^{\cdot -}$) are constantly formed in all aerobic cells. Low levels of endogenous ROS are essential to the cells for regulating various physiological processes such as cell signaling pathways and regular cell proliferations (Burdon et al., 1989). However, an excess production of ROS in cells under various pathological conditions can cause mitochondrial dysfunctions, protein oxidation, DNA mutations and excessive cellular damage, all of which can lead to translocation of cyt *c* from mitochondria to cytosol. The translocation of cyt *c* from mitochondria to cytosol is a critical event in the activation of intracellular signaling; it results

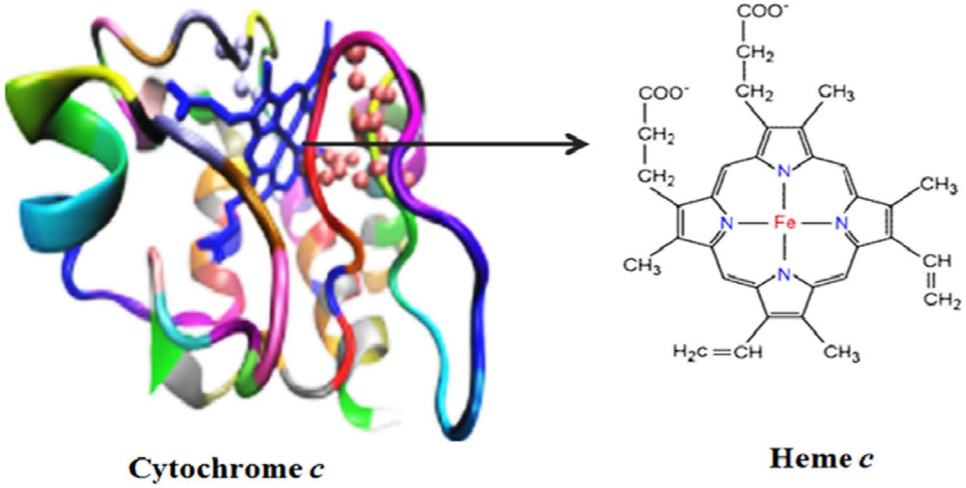

Fig. 1. Cyt *c* contains one heme prosthetic group inserted into a hydrophobic cleft in the protein.