Analytica Chimica Acta 842 (2014) 20–26

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Electrochemical monitoring of intracellular enzyme activity of single living mammalian cells by using a double-mediator system

Yoshiharu Matsumae [a], Yasufumi Takahashi [b], Kosuke Ino [a], Hitoshi Shiku [a,*], Tomokazu Matsue [a,b,**]

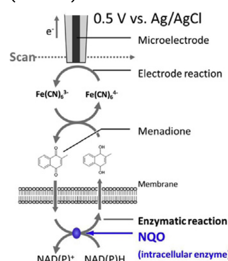
[a] Graduate School of Environmental Studies, Tohoku University, Aramaki 6-6-11-605, Aoba, Sendai 980-8579, Japan
[b] Advanced Institute for Materials Research, Tohoku University, Katahira 2-1-1, Aoba, Sendai 980-8577, Japan

HIGHLIGHTS

- NAD(P)H:quinone oxidoreductase activity of single cells were evaluated with SECM.
- $Fe(CN)_6^{3-}$/menadione concentrations were optimized for long-term SECM monitoring.
- Menadione affect the intracellular levels of reactive oxygen species and GSH.
- At 100 μM menadione, the $Fe(CN)_6^{3-}$ generation rate decreased rapidly within 30 min.

GRAPHICAL ABSTRACT

NAD(P)H:quinone oxidoreductase (NQO) activity of single HeLa cells were evaluated by using the menadione–ferrocyanide double mediator system combined with scanning electrochemical microscopy (SECM).



ARTICLE INFO

Article history:
Received 8 April 2014
Received in revised form 25 June 2014
Accepted 27 June 2014
Available online 28 June 2014

Keywords:
Scanning electrochemical microscopy
Menadione
Glutathione
Reactive oxygen species
Ferricyanide

ABSTRACT

We evaluated the intracellular NAD(P)H:quinone oxidoreductase (NQO) activity of single HeLa cells by using the menadione–ferrocyanide double-mediator system combined with scanning electrochemical microscopy (SECM). The double-mediator system was used to amplify the current response from the intracellular NQO activity and to reduce menadione-induced cell damage. The electron shuttle between the electrode and menadione was mediated by the ferrocyanide/ferricyanide redox couple. Generation of ferrocyanide was observed immediately after the addition of a lower concentration (10 μM) of menadione. The ferrocyanide generation rate was constant for 120 min. At a higher menadione concentration (100 μM), the ferrocyanide generation rate decreased within 30 min because of the cytotoxic effect of menadione. We also investigated the relationship between intracellular reactive oxygen species or glutathione levels and exposure to different menadione concentrations to determine the optimal condition for SECM with minimal invasiveness. The present study clearly demonstrates that SECM is useful for the analysis of intracellular enzymatic activities in single cells with a double-mediator system.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

The intracellular enzymatic activity of a single living cell is a beneficial indicator of its metabolic vitality. Novel analytical tools permitting low-invasive, continuous, quantitative, and single-cell

Abbreviations: RG, the ratio of the insulating glass sheath radius to the disk electrode radius.

* Corresponding author. Tel.: +81 22 795 6167; fax: +81 22 795 6167.
** Corresponding author. Tel.: +81 22 795 7209; fax: +81 22 795 7209.
E-mail addresses: shiku@bioinfo.che.tohoku.ac.jp (H. Shiku), matsue@bioinfo.che.tohoku.ac.jp (T. Matsue).

http://dx.doi.org/10.1016/j.aca.2014.06.047
0003-2670/© 2014 Elsevier B.V. All rights reserved.

level measurements to estimate the intracellular enzymatic activity are required. Electrochemical methods have enabled the noninvasive quantitative analysis of redox enzyme activity by using mediators that shuttle electrons between the electrode and enzymes. In particular, hydrophobic redox mediators are known to undergo transmembrane diffusion processes and can be utilized to investigate intracellular redox activity [1,2]. In addition, scanning electrochemical microscopy (SECM), which is a scanning probe microscopy technique and uses a micro- or nano-electrode as a probe, is suitable to measure enzymatic activities with high spatial resolution (less or equal to the size of a single cell) [3,4]. Because SECM is useful to image and analyze the efflux or uptake of redox species from a sample surface, it has been used for single-cell measurements of stress-related chemicals such as neurotransmitters [5–7], nitric oxide [8], reactive oxygen species (ROS) [9,10], and oxygen [11–13]. Intracellular and cell surface enzyme activities have also been detected with minimal invasiveness and characterized quantitatively [14–21].

Menadione is a quinone derivative with high cell membrane permeability and has been used to evaluate intracellular enzyme activities [14–16,22,23]. However, it is also known that menadione is cytotoxic. In the cell, menadione is detoxified by the NAD(P)H:quinone oxidoreductase (NQO) (EC 1.6.5.2) or eliminated by the conjugation reaction with glutathione (GSH) [24]. The NQO is a key enzyme that provides protection from quinone species. NQO protects against the deleterious effects of quinones by catalyzing their two-electron reduction without generating radical species. In general, quinones can readily undergo one-electron reduction reactions by other reductases and this process generates ROS, which are extremely cytotoxic because they induce lipid peroxidation, enzyme inactivation, and modification of DNA. This reaction also results in the generation of reactive semiquinone intermediates that can form adducts directly with cellular macromolecules, including DNA, thereby making them carcinogenic [25]. Another detoxification mechanism is the formation of a menadione conjugate with the reduced form of GSH. GSH is the major endogenous antioxidant, preventing damage of important cellular components from ROS attack. After the conjugation reaction of menadione with GSH, the complex is pumped out of the cell. Bard et al. detected the GSH–menadione complex in yeast cells [15] and Hep G2 cells (a human liver carcinoma cell line) [14] and calculated the efflux rate using on a constant-flux model.

The menadione–ferrocyanide double-mediator system has been used to amplify the current response from intracellular NQO activities (Fig. 1) [22,23,26]. Ferricyanide helps to overcome the slow heterogeneous redox kinetics of menadione on Pt electrodes, which results in a current increase. The current was also amplified by redox cycling of the ferricyanide/ferrocyanide turnover between the detector electrode and the menadione/reduced form of menadione couple. Nagamine et al. previously reported evaluation of the NQO activities of yeast cells by this system with SECM [16], and they optimized menadione concentration to maximize current responses. However, owing to menadione cytotoxicity, that condition is not suited for the time-dependent measurements. Conversely, there is no concentration limit on ferrocyanide because of its very low cell membrane permeability. If the ferricyanide concentration is much higher than that of menadione, it is not possible to detect menadione directly on the electrode because almost all menadione is reoxidized by ferricyanide near the cellular surface. If we assume that the concentrations of menadione and ferricyanide are in large excess over the value of the Michaelis constant of NQOs and that the microelectrode detects the diffusion-limited current, the rate-limiting step for the determination of the current response is the intracellular NQOs reaction [27].

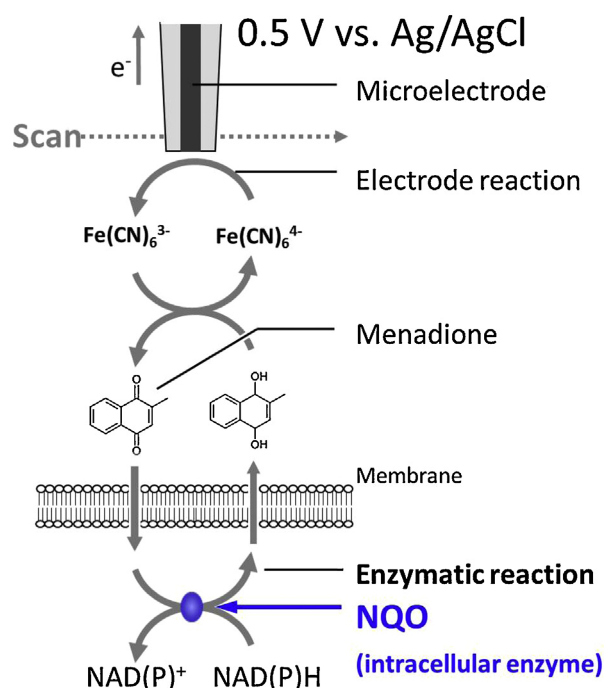


Fig. 1. Principle of electrochemical detection of NAD(P)H:quinone oxidoreductase (NQO) activity in a HeLa cell. $Fe(CN)_6^{3-}$ and menadione were added in the measurement solution.

In this study, the NQO activity of single living cells was monitored electrochemically with a double-mediator system and SECM at different concentrations of menadione as a membrane-permeable (hydrophobic) mediator. Ferricyanide, as a non-permeable mediator, was used for the highly sensitive detection of NQO activity. We optimized menadione concentration to minimize cytotoxicity and optimized ferricyanide concentration to obtain clear response. We also determined the amount of intracellular ROS and GSH at different menadione concentrations.

2. Materials and methods

2.1. Chemicals and materials

Potassium hexacyanoferrate (III), menadione, D-(+)-glucose, and 4-(2-hydroxyethyl)-L-piperazine-1-ethanesulfonic acid (HEPES) were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). All solutions were prepared using distilled and deionized water (Direct-Q, Millipore).

2.2. Cell culture

HeLa cells were provided by the Cell Resource Center for Biomedical Research (Tohoku University, Sendai, Japan). They were cultured on plastic petri dishes (Ø: 35 mm) in RPMI-1640 medium (Gibco Invitrogen, Tokyo, Japan) supplemented with 10% fetal bovine serum (Gibco) and 1% penicillin/streptomycin (Gibco) at 37 °C in a humidified atmosphere containing 5% CO_2. For single-cell measurements, HeLa cells were seeded on a 35 mm dish at a low concentration (1×10^3 cells per dish). After incubation for 1 day, these cells were used for SECM measurements.

2.3. SECM set-up

A disk-type Pt electrode with a diameter of 20 μm (RG = 2, RG, the ratio of the insulating glass sheath radius to the disk electrode

Download English Version:

https://daneshyari.com/en/article/7555734

Download Persian Version:

https://daneshyari.com/article/7555734

Daneshyari.com