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Electrochemical monitoring of native catalase activity in skin using skin covered oxygen electrode

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ABSTRACT

A skin covered oxygen electrode, SCOE, was constructed with the aim to study the enzyme catalase, which is part of the biological antioxidative system present in skin. The electrode was exposed to different concentrations of H_2O_2 and the amperometric current response was recorded. The observed current is due to H_2O_2 penetration through the outermost skin barrier (referred to as the stratum corneum, SC) and subsequent catalytic generation of O_2 by catalase present in the underlying viable epidermis and dermis. By tape-stripping the outermost skin layers we demonstrate that SC is a considerable diffusion barrier for H_2O_2 penetration. Our experiments also indicate that skin contains a substantial amount of catalase, which is sufficient to detoxify H_2O_2 that reaches the viable epidermis after exposure of skin to high concentrations of peroxide (0.5–1 mM H_2O_2). Further, we demonstrate that the catalase activity is reduced at acidic pH, as compared with the activity at pH 7.4. Finally, experiments with often used penetration enhancer thymol shows that this compound interferes with the catalase reaction. Health aspect of this is briefly discussed. Summarizing, the results of this work show that the SCOE can be utilized to study a broad spectrum of issues involving the function of skin catalase in particular, and the native biological antioxidative system in skin in general.

1. Introduction

Since the invention of biosensors in 1962 (Clark and Lyons, 1962), the Clark oxygen electrode has been used to construct biosensors based on various enzymes, cells, and tissues (Turner et al., 1987). In most cases, the mentioned biological materials served as a recognition element providing specificity for the transduction function of the biosensor. In several cases the biosensor design has been used to address functions of the biomaterials itself, e.g., to monitor the activity of surface bound enzymes (Haberska et al., 2008; Ruzgas et al., 1995) or yeast cells attached at the electrode (Heiskanen et al., 2009; Spégel et al., 2007). Recently we proposed a skin covered electrode for studies of dynamics of transdermal penetration of biologically active compounds such as hydrogen peroxide, ascorbic acid, and quercetin (Gari et al., 2015; Rembiesa et al., 2015).

In this work we assessed the possibility of covering an oxygen electrode with excised pig skin membranes for investigation of native catalase function in skin. Catalase is one of the enzymes of the biological antioxidative system in skin (Pillai et al., 2005) where it removes hydrogen peroxide, H_2O_2, from skin by converting it to water and oxygen according to:

$$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2 \quad (1)$$

H_2O_2 is one of the most stable forms of reactive oxygen species, ROS, which might be generated in skin by both exogenous and endogenous factors. Exogenous factors leading to elevated ROS in skin is, for example, exposure to UV irradiation, whereas chronic inflammation is an example of endogenous factor. Considering the general relevance of these examples, it is clear that monitoring the skin´s ability to detoxify ROS is of great interest in a wide range of situations including healthcare settings and development of topical products such as sun screen lotions. To the best of our knowledge, this work represents the first study where it is demonstrated that the oxygen electrode can be utilized for electrochemical in-vitro monitoring of native catalase activity in skin. The proposed electrode design is a first step towards a simple and easy operated electrochemical tool to assess the function of native antioxidative system in skin.

As a background to help appreciate the results of this work, the skin

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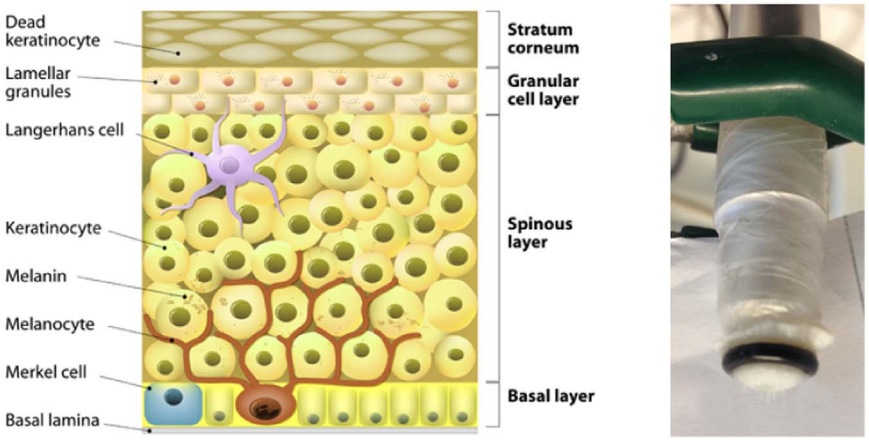

Fig. 1. (left) Schematic presentation of layered structure of skin membrane consisting of stratum corneum (SC) and viable epidermis (Creative Commons (Attribution 3.0) from freedesignfile.com) and (right) a photo of oxygen electrode covered with skin membrane.

structure is briefly described by the schematic drawing in Fig. 1a. It should be kept in mind that the structure of the skin is much more complicated, however, our simplified description is sufficient to understand the results of this work. Skin is one of the largest organ of the body and consists of several anatomically different layers as seen in Fig. 1a. The outermost layer is called the stratum corneum, SC, and is usually represented by the brick and mortar model, where the bricks represent dead cells (i.e. corneocytes), which are surrounded by a continuous lipid multilamellar matrix, i.e. the mortar (Michaels et al., 1975). Tape-stripping of the SC is often used to demonstrate that the barrier properties of the skin for transdermal penetration vanishes when the SC is stripped of (Valk and Maibach, 1990). In particular, the continuous lipid lamellae matrix of SC represents a tough diffusional barrier towards transdermal penetration and thus ensures hydration homeostasis by minimizing the water loss from the body to the external environment (Iwai et al., 2012; Scheuplein and Blank, 1971). Interestingly, the transdermal permeability of the skin barrier can be reversibly regulated by changing the degree of SC hydration (Björklund et al., 2010, 2013). This example emphasizes the fact that the skin is a responding membrane, i.e., the membrane can change its properties following the changes of external biophysical factors (Björklund et al., 2010). Regulation of transdermal permeability as well as monitoring of enzyme activity in skin are, thus, of broad medical and industrial interest.

The results described in this work provide a number of important conclusions. Firstly, we demonstrate that the activity of native catalase in skin is considerable in freshly excised pig skin membranes and that it is possible to switch-off the catalase activity in skin by an enzyme inhibitor. Secondly, the catalase activity is high enough to detoxify transdermal penetration of H_2O_2 from a relatively concentrated solution of H_2O_2. Thirdly, from tape-stripping experiments we conclude that the catalase is primarily located in the viable epidermis and that the H_2O_2 penetration is significantly enhanced upon successive stripping of SC. Fourthly, the activity of native catalase is severely suppressed by changing the pH from neutral to acidic solution in contact with the SC barrier.

Taken together, several of the findings in the present study are important to consider when developing topical formulations. For example, when the skin becomes exposed to UV irradiation, i.e., the conditions when ROS and H_2O_2 production might be elevated and functioning of catalase in skin should not be compromised. Our experiments also indicate that some penetration enhancers, i.e., components of topical formulations, can interfere with catalase reaction in skin.

2. Material and methods

2.1. Material

Hydrogen peroxide (30%, 10.3 M), phosphate buffer saline (PBS, pH 7.4) in tablets, sodium citrate and sodium chloride for preparation of citrate buffer saline (CBS, pH 4.0 consisting of 10 mM sodium citrate and 150 mM NaCl), thymol, and azide were purchased from Sigma Aldrich. Oxygen electrode, constructed using 5 μm thick Teflon membrane from DuPont Fluoropolymers (Detroit, MI, USA), 250 μm Pt melted in glass and internal Ag/AgCl reference, was purchased from UAB "OPTRONIKA", Vilnius, Lithuania. All solutions have been prepared by using deionised water purified by Milli-Q system (Merck Millipore, Billerica, USA) with resistivity of 18.2 Ω cm.

2.2. Preparation of skin membranes

Fresh pig ears were obtained from a local abattoir and stored at −80 °C if not immediately used. To prepare skin membranes, fresh or defrosted pig ears were rinse with cold water and cut into strips with a scalpel. Outer part (stratum corneum side) of the strip was shaved and approximately 500 μm thick skin membranes were sliced with a dermatome. The resulting skin stripes were punched out to make circular membranes with 16 mm in diameter. If not immediately mounted on the oxygen electrode, the membranes were kept in the fridge (+4 °C) on a filter paper soaked with PBS. Skin membranes, prepared as described, were used within two weeks.

2.3. Preparation of skin covered oxygen electrode (SCOE)

The platinum cathode surface of the oxygen electrode was polished using alumina suspension (1 μm alumina, Buehler, Lake Bluff, IL) and rinsed with deionized water. The body of the electrode was filled with saturated KCl solution and covered with 5 μm Teflon membrane. This constituted the Clark type oxygen electrode. This electrode was then covered with the skin membrane as shown in Fig. 1b.

2.4. Amperometric monitoring of catalase activity in skin

The skin membrane covered oxygen electrode (Fig. 1b) was dipped into an electrochemical cell filled with 20 mL PBS (pH 7.4) or CBS (pH 4.0) buffer. Electrochemical measurements were performed using a CompactStat potentiostat from IVIUM Technologies (Eindhoven, The Netherlands). The oxygen electrode was connected to the potentiostat in two electrode configuration by applying −0.7 V vs Ag/AgCl/KClsat on Pt cathode of the oxygen electrode. After the baseline current was stabilized, a defined amount of H_2O_2 was pipetted into the electro-