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Molecular Identification through Membrane Engineering as a revolutionary concept for the construction of cell sensors with customized target recognition properties: the example of superoxide detection

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Abstract

Membrane-engineering is a generic methodology for increasing the selectivity of a cell biosensor against a target molecule, by electroinserting target-specific receptor molecules on the cell surface. We have previously reported the construction of an ultra-sensitive superoxide anion ($O_2^{\bullet -}$) sensor based on immobilized cells, which have been membrane-engineered with superoxide dismutase (SOD). In the present study, we provide evidence that superoxide dismutation triggered changes to the membrane potential of membrane-engineered fibroblast cells, as confirmed by electrophysiological and fluorescence assays. In addition, by conducting selective inhibition assays, we show that electroinserted SOD molecules retained their characteristic catalytic properties. We also investigated the effect of the concentration of electroinserted SOD molecules. Finally, we increased the sensitivity of the sensor by hundredfold to a detection limit of 1 pM $O_2^{\bullet -}$ by changing the intensity of the electrical field during electroinsertion and the concentration of immobilized cells on the performance of the biosensor.

Keywords: Cell biosensor; Immobilization; Membrane engineering; Superoxide anion; Bioelectric Recognition Assay

1. Introduction

A cell-based sensor design employs the physiological responses of whole living cells as the sensing component. Therefore, they are able to provide physiologically relevant data in response to an analyte and to measure the bioavailability of the analyte [1]. One of the traits that make cell biosensors attractive as a clinical analytical tool is their considerable sensitivity. But the problem is that since cells can react in roughly the same manner against an amazingly large number of different molecules, cell sensors can exhibit a very poor selectivity.

In recent years a number of cell transfection methods have been developed for increasing cell specificity, with considerable success [2-5]. However, the applicability of cellular transfection is limited by the lack

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of stability and the frequent, unwanted alteration of cellular phenotype. We previously reported [6] the first application of this technology for the construction of an ultra-sensitive electrophysiological superoxide sensor, which was based on "membrane-engineered" mammalian cells immobilized in an alginate matrix. The membrane-engineering process involved the electroinsertion of superoxide dismutase (SOD) molecules in the membranes of Vero fibroblast cells, which acted as catalytic units able to convert $O_2^{\bullet-}$ to H_2O_2. The sensor instantly responded to picomole concentrations of $O_2^{\bullet-}$ with a detection limit of 100 pM.

2. Results and discussion

2.1 Inhibition of SOD abolishes the interaction between superoxide and membrane-engineered cells

Vero cells, which have been membrane-engineered with SOD [6] and were incubated in 3,3 dipropylthiadicarbocyanide iodide emitted a bright red fluorescence, corresponding to their steady-state membrane potential under the applied experimental conditions. The intensity of fluorescence was changed in response to the presence of 1 nM $O_2^{\bullet-}$ in a pattern indicating increased cell membrane hyperpolarization (Fig. 1a). Control cells (electroporated but not membrane-engineered with SOD) demonstrated a decrease of the membrane potential, possibly due to superoxide-mediated membrane lipid oxidation and reduction of membrane function [7]. However, considerably lower changes in membrane potential were observed when cells were treated with either 2 mM NaCN or 2 mM H_2O_2, two well-known SOD inhibitors, compared to membrane-engineered cells with SOD. The superoxide-induced increase of the membrane potential in membrane-engineered cells was associated with a considerable increase of cytosolic Ca^{2+} concentration, as measured by staining with Fluo-3 (Fig. 1b). A lower increase was observed in control cells (electroporated but not membrane-engineered with SOD). Changes in calcium ion concentrations have been previously reported [8,9], as possible mechanism accompanying the interaction between electroinserted receptors and their homologous analytes. Also, Whelan and Zare [10] have previously shown that receptor-like interactions between molecules on the cell surface and target analytes resulted in a detectable change in the concentration of cytosolic Ca^{2+}.

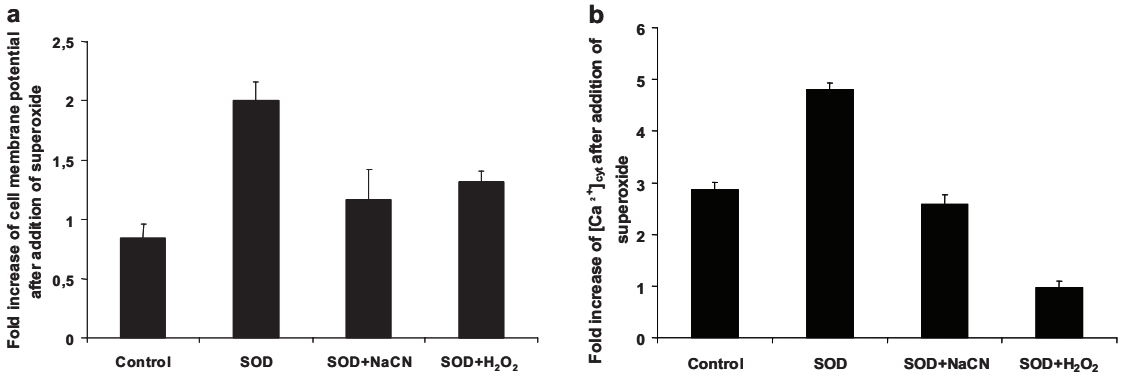

Fig. 1: Changes induced by 1 nM $O_2^{\bullet-}$ (expressed as fold increase in fluorescence intensity relative to non-induced cells with $O_2^{\bullet-}$) of the cell membrane potential (a) or the $[Ca^{2+}]_{cyt}$ (b) of membrane-engineered cells with SOD (SOD) or only electroporated (control). Prior to the addition of superoxide, cells were untreated or treated with 2 mM NaCN (SOD+NaCN), inhibitor or with 2 mM H_2O_2 (SOD+H_2O_2), CuZnSOD inhibitors. Data correspond to the mean±S.E.M.

2.2 Sensor sensitivity is increased by increasing the concentration of electroinserted SOD molecules

In the absence of superoxide (control samples), the sensor response increased when the concentration of electroinserted SOD was increased (Fig. 2). This might have been due to increased cell membrane porosity resulting from the membrane-engineering process (since electroinsertion is a variation of electroporation). Exposure of cell membranes to electric fields can cause lipid re-arrangement resulting to the creation of conductive membrane pathways, known as "hydrophilic" or "conductive" pores [8,11]. When cells were membrane-engineered with 750 units mL^{-1} SOD, the

resulting sensors did not demonstrate a significantly different response to the addition of 0.1 nM superoxide; a slight decrease of the sensor potential was observed by adding superoxide at 1 or 10 nM concentration. On the contrary, by increasing the concentration of electroinserted SOD to 1500 or 3000 units mL^{-1}, a significantly high sensor response to 0.1 nM superoxide was observed. Similarly to the electroinsertion of 750 units mL^{-1} SOD, the sensor response declined at higher superoxide concentrations (1 or 10 nM).

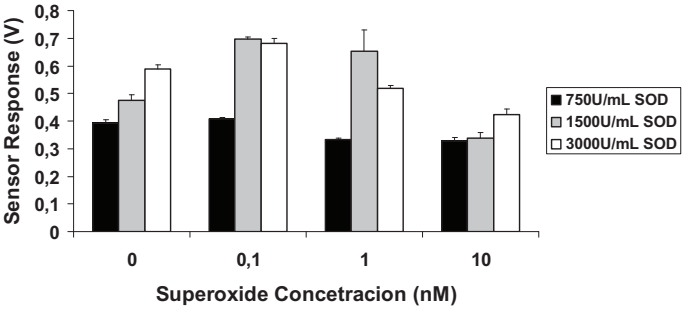

Fig. 2: Sensor response to different superoxide concentrations after electroinserting SOD molecules at different concentrations in membrane-engineered cells. Sensor response is expressed as a change in the membrane potential of immobilized cells. Concentration of electroinserted SOD (units mL^{-1}): *black columns* 750, *grey columns* 1500, *white columns* 3000. Presented values correspond to average sensor response

2.3 Sensor response depends on both cell density and the conditions of electroinsertion

Both the intensity of the electric field during electroinsertion and the final density of immobilized Vero-SOD in the sensor affected the sensor's performance. When electroinsertion was conducted at a field intensity of 400 V cm^{-1}, the sensor's steady-state potential was slightly increased by increasing cell density from 50 x 10^3 to 100 x 10^3 cells/sensor (Fig. 3a). However, only sensors containing Vero-SOD cells at the lowest density (50 x 10^3) responded significantly (i.e. higher than control) to 1-10 pM superoxide, whereas sensors with immobilized cells at the highest density (100 x 10^3) responded only to 50 pM superoxide. A different pattern was observed when electroinsertion was conducted at a field intensity of 1800 V cm^{-1}. In this case, the sensor's steady-state potential was significantly higher when cells were immobilized at an intermediate density of 75 x 10^3/sensor (Fig. 3b) and only sensors containing immobilized cells at the intermediate or highest density (75-100 x 10^3) responded significantly higher to 1 pM $O_2^{\bullet-}$. Maximum sensor response (against 100 pM $O_2^{\bullet-}$) was recorded from sensors with cells at the intermediate density of 75 x 10^3. However, this response was not significantly different from the sensor's response against 1 pM $O_2^{\bullet-}$, due to the considerable variation in the sensor's potential at this particular superoxide concentration (100 pM).

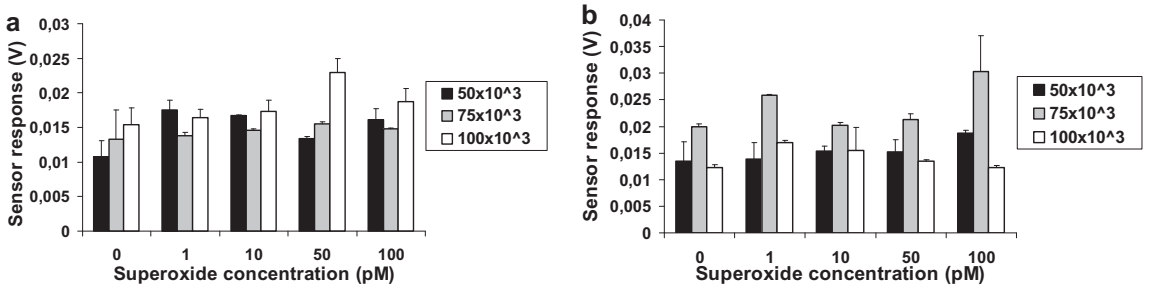

Fig. 3: Effect of different electroinsertion conditions (electric intensities) (a: 400 V cm^{-1}, b: 1800 V cm^{-1}) and cell densities on the sensor response to different superoxide concentrations. Sensor response is expressed as a change in the membrane potential of immobilized cells. Density of immobilized cells (cells $sensor^{-1}$): *black columns* 50 x 10^3, *grey columns* 75 x 10^3, *white columns* 100 x 10^3. Presented values correspond to average sensor response.

Differences in electric field strength during electroinsertion may have affected the catalytic properties of membrane-engineered cells in various ways. Higher field intensities have been associated with increased thermal effects due to Joule heating [11], while lower field strengths have been associated with increasing non-uniform cell membrane hyperpolarization [12].

3. Conclusion

Membrane-engineering is a very recent approach for the construction of cellular biosensors with designed selective responses against different analytes. Based on the results of the present study, we can draw the following preliminary conclusions about the properties of electroinserted SOD molecules and the superoxide-catalyzing properties of membrane-engineered cells:

- As reported previously [6], membrane-engineered cells acted as catalytic units able to convert $O_2^{\bullet -}$ to H_2O_2. Moreover, electroinserted SOD molecules retained their characteristic properties, as demonstrated by the selective inhibition assays.
- The catalytic properties of the membrane-engineered cells could be increased by increasing the concentration of electroinserted SOD molecules.
- Depending on the conditions of electroinsertion, increasing the concentration of immobilized cells could lead to a considerable increase of the sensor sensitivity down to 1 pM $O_2^{\bullet -}$ (a hundredfold increase compared to the previously reported detection limit of 100 pM).

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