IJABPT
INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY
www.ijabpt.com
ISSN : 0976-4550
Volume-7, Issue-2, April-June-2016 Coden IJABFP-CAS-USA
Received: 10th Jan 2016 *Revised: 26th Jan 2016* *Accepted: 02nd Feb 2016*
Short Communication

DENITRIFICATION ENZYMES-BASED BIOSENSORS: THE CASE OF HALOARCHAEAL ENZYMES

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ABSTRACT: In the last two decades, the increase in the use of artificial fertilizers and the disposal of industrial wastes have been the main factors responsible for the progressive increase in nitrate and nitrite levels in groundwater and soil. A variety of analytical strategies have been developed for nitrate and nitrite detection but electrochemical biosensors, which are simple, cheap, easily miniaturized and suitability for real-time detection, are proved to be a powerful tool. Various types of biosensors based on the use of whole cells or on the immobilization of denitrification enzymes have been developed, but their use is limited in environmental analysis under extreme conditions such as brines, acidic or basic wastewaters, salted soils, etc. Extremophilic denitrifying microorganism are good candidates for the development of new nitrate and nitrite biosensors and, in particular, haloarchaeal based biosensors would have advantages over bacterial based biosensors since the microorganisms and the purified denitrifying enzymes tolerate a wide range of temperature and salinity. This work summarizes new highlights on the potential uses of denitrifying haloarchaeal enzymes to make enzyme-based biosensors.
Key words: Biosensors; Enzymes; Denitrification; Respiratory nitrate reductase; Respiratory nitrite reductase

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INTRODUCTION

The term "biosensor" refers to a device that incorporates a biological sensing element connected to a transducer. The aim of these devices is to convert an observed response into a measurable signal, whose magnitude should be proportional to the concentration of a specific chemical or set of chemicals to be quantified (Eggins, 1996; Eggins, 2002; Zhao & Jiang, 2010). Biosensors can be classified into different categories following different criteria (Rodriguez-Mozaz et al., 2006; Slonczewski et al., 2010):

- On the basis of the bio-recognition principle, biosensors are classified into antibodies and antigens, enzymatic, non-enzymatic, whole-cell and nucleic acids biosensors (Karube & Suzuki, 1986; Marazuela & Moreno-Bondi, 2002; Rodriguez-Mozaz et al., 2006).
- According to the transduction process, biosensors can be divided into electrochemical, optical, piezoelectric, and thermal/calorimetric categories.

Among these various kinds of biosensors, electrochemical enzyme-based biosensors are one of the most widespread and successfully commercialized devices of biomolecular electronics (Dzyadevycha et al., 2008).

In that case, enzymes can be immobilized by following different methods: adsorption, entrapment, covalent binding and cross-linking (Zhao & Jiang, 2010). Enzyme-based electrochemical biosensors have been used widely in our society to improve health care, food safety and environmental monitoring. Health care and medical diagnosis are probably the main areas for biosensor applications (Wilkins, 1989), although other relevant industrial applications for biosensors include monitoring in food processing, food quality control or environmental quality (contamination monitoring), as mentioned before. The history of biosensors is quite new, but highly innovative. The first scientific manuscript focused on biosensors was published in the early sixties, last century. From that date up to now, more than 40.000 research papers have summarized optimization, innovation and new potential uses of biosensors (http://www.ncbi.nlm.nih.gov/pubmed; "biosensor" as key word to carry out the search). This amazing scientific production (in just half century) clearly reveals how important biosensors are to contribute to well-being.

In this work, new highlights on denitrification enzyme based biosensors are summarized paying special attention to denitrification enzymes isolated from halophilic archaea (microorganism requiring high salt concentration to be alive).

ENVIRONMENTAL CONTAMINATION BY NITRATES AND NITRITES

Anthropogenic activities are dramatically affecting the nitrogen cycle, one of the most important biogeochemical cycles in nature (Martínez-Espinosa et al., 2011). During the last decades, legislative actions as well as environmental policies are focused on environmental pollution control (Rodriguez-Mozaz et al., 2006). Special attention has received soil and water quality control in terms of nitrates and nitrites concentrations due to their negative effects on human health. The main governmental agencies all around the world have promulgated rules and directives to restrict the level of these ions in drinking water and food products (Almeida et al., 2010). Although some positive effects of nitrate of human health have been reported (Kuennen et al., 2015; Bakker et al., 2015), nitrate and nitrite consumption by animals in general, and by humans in particular, promote methaemoglobinaemia and cancer (Kross et al., 1992; Saigal et al., 2014; Sowjanya et al., 2015; Drozd et al., 2015; Song et al., 2015; Inoue-Choi et al., 2015).

In order to comply with the new controls and new social requirements, nitrates and nitrites measurements should be quick, accurate and efficient. The quantification of nitrates and nitrites using traditional analytical methods such as chromatography, spectrophotometry or polarography is expensive and time consuming. On the other hand, these kinds of quantifications have susceptibility to matrix interferences and usually they require pre-treatments (Cosnier et al., 2008; Almeida et al., 2010).

Taking into account all the previous reasons and paying special attention to environmental quality monitoring, it becomes necessary to develop systems which can detect a lot of compounds in environmental samples as quickly and as cheaply as possible (Rodriguez-Mozaz et al., 2006). Another aspect to be considered is that some of the environmental analysis done in situ requires devices able to measure parameters under extreme conditions (high or low pH's, extreme temperatures, high ionic strength, etc.). Due to these reasons, enzyme-based biosensors become one of the most important devices, especially those containing extreme-enzymes able to catalyze reactions under extreme environmental conditions.

BIOSENSORS BASED ON DENITRIFICATION ENZYMES

Denitrification is a metabolic pathway carried out by some microorganisms, in which nitrate is fully reduced to dinitrogen (Martínez-Espinosa et al. 2011). This metabolic pathway is catalyzed by four enzymes: respiratory nitrate reductase (it reduces nitrate to nitrite), respiratory nitrite reductase (it reduces nitrite to nitric oxide), nitric oxide reductase (it reduces nitric oxide to nitrous oxide) and nitrous oxide reductase (which reduces nitrous oxide to dinitrogen) (Martínez-Espinosa et al. 2011).

Due to the importance of nitrates and nitrites as contaminants in soils and waters, two main lines of biosensors are emerging: i) biosensors based on the use of whole cells (the biosensor detects products of cellular nitrogen metabolism), ii) systems based on the immobilization of denitrification enzymes in a matrix. In the first case, specific nitrate and nitrite (NOx–) biosensors for environmental analysis using bacterial cells have been described (Larsen et al., 1997; Larsen et al., 2000; Andersen et al., 2001; Nielsen et al., 2009).

The cells are placed in a reaction chamber where the reduction NO_3^- to N_2O occurs and it is measured by a specific nitrous oxide micro-electrode (the strains used in the NOx- biosensors have a truncated denitrifying pathway ending at N_2O instead of N_2). For the specific nitrite determination, a sensitive NO_2- biosensor has been developed using four denitrifying organisms lacking nitrate reductase activity (Nielsen 2004). Modified cell biosensors are being constructed by fusing a reporter gene to a promoter element that is induced by the presence of a target compound. In this sense, a whole-cell fluorescence biosensor based on recombinant Escherichia coli allowed the determination of nitrate without the interference of phosphate, chloride and nitrite (Taylor et al., 2004).

In the second case, the enzymes (nitrate and nitrite reductase, mainly) isolated from denitrifying bacteria such as species of the Paracoccus, Alcaligenes and Desulfovibrio genera, are immobilized in different materials to improve their stability and half-life (Glazier et al., 1998; Cui et al., 2006; Cosnier et al., 2008; Almeida et al., 2010; Plumeré, 2012; Mohd Zuki et al., 2014; Madasamy et al., 2014; Siontorou et al., 2016). In most of these biosensors, redox mediators (viologen derivatives) have to be used to shuttle electrons from the protein redox centres to the transducing elements (ammeters or voltmeters), although direct electron transfer between the enzymes and the electrode material are also possible.

One of the major problems that these biosensors have is that the whole cells or the isolated enzymes should work under specific environmental conditions that promote high stability and high enzymatic activity (temperatures around 20-30°C, neutral pH's, low ionic strength, etc.). Consequently, those biosensors are not useful to quantify nitrate and nitrite in environmental samples such as brines, acidic or basic wastewaters, salted soils, etc.

In that context, extremophilic denitrifiers are good candidates to make innovate biosensors. Those microorganisms are able to perform reactions under very hostile conditions (high or low temperatures, extreme pH's, high ionic strength, etc.). At the time of writing this work, there is only a study focused on the use of a psychotropic bacteria based NO_x– biosensor to analyze marine sediments. This biosensor can be used at low temperature (<2.5°C) and high salinity (35%) (Revsbech and Glud, 2009). Several studies about nitrogen cycle in haloarchaea (extremophilic microorganisms requiring high salt concentrations to be alive) suggest that some denitrifying haloarchaea are highly efficient removing nitrates and nitrites from salted water (Martínez-Espinosa et al., 2006; Nájera-Fernández et al., 2012). So, new biosensors could be developed using whole haloarchaeal cells or even isolated nitrate and nitrite reductases. Respiratory nitrate and nitrite reductases from Hfx. *Mediterranei* have been isolated and characterized into the detail (Lledó et al., 2004; Esclapez et al., 2013). On the basis of their biochemical properties, haloarchaeal enzyme-based-biosensors would have advantages over bacterial enzyme-biosensors described since today due to the following reasons: i), nitrate and nitrite reductases from this haloarchaea tolerate high salt concentrations, allowing their use in salt water and brines (Lledó et al., 2004; Martínez-Espinosa et al., 2007); ii), their Km values are quite low comparing to bacterial homologues, so the affinity for their substrates is very high (Km for nitrite in the case of Hfx. *Mediterranei* nitrite reductase (NirK) is 4.04 ± 0.33 mM which is almost 100-fold greater than for other NirK characterized) (Esclapez et al., 2013), iii) some haloarchaeal nitrate reductases are also able to remove (per)chlorate, which is also another contaminant frequently present in those environments where nitrate is a contaminant (Martínez-Espinosa et al., 2015).

Despite all the mentioned advantages, it has not developed any prototype of biosensor for *Haloferax* yet. So new research lines should be addressed in that sense in the next future.

CONCLUSIONS

Currently, there are many challenges facing towards practical applications of biosensors, mainly for environmental monitoring under slightly or even extreme conditions in terms of temperature, pH, ionic strength, etc. The construction of a biosensor able to work under extreme conditions with a low cost is still essential when considering the commercial devices. Other aspects such as miniaturization, portability and multifunction analysis should be integrated. Challenges also exist to find ways to improve the performance criteria including high sensitivity, wider linear range, and low limit of detection, fast response and repetitive ability. Research work now still keeps continuing to investigate more effective ways to construct extreme enzyme-based electrochemical biosensors with more perfect performance.

ACKNOWLEDGEMENTS

This work was funded by research grant from the MINECO Spain (CTM2013-43147).

REFERENCES

Almeida MG, Serra A, Silveira CM, Moura JJ (2010). Nitrite biosensing via selective enzymes--a long but promising route. Sensors (Basel) 10: 11530-11555.

Bakker E, Engan H, Patrician A, Schagatay E, Karlsen T, Wisløff U and Gaustad SE (2015). Acute Dietary Nitrate Supplementation Improves Arterial Endothelial Function at High Altitude: A Double-Blinded Randomized Controlled Cross over Study. Nitric Oxide: Vol.29, 50, 58-64.

Cosnier S, Da Silva S, Shan D, Gorgy K (2008). Electrochemical nitrate biosensor based on poly(pyrrole-viologen) film-nitrate reductase-clay composite. Bioelectrochemistry 74: 47-51.

Cui Y, Barford JP, Renneberg R (2006). Development of a bienzyme system for the electrochemical determination of nitrate in ambient air. Anal Bioanal Chem 386: 1567-1570.

Drozd VM, Saenko VA, Brenner AV, Drozdovitch V, Pashkevich VI, Kudelsky AV, Demidchik YE, Branovan I, Shiglik N, Rogounovitch TI, Yamashita S, Biko J and Reiners C (2015). Major Factors Affecting Incidence of Childhood Thyroid Cancer in Belarus after the Chernobyl Accident: Do Nitrates in Drinking Water Play a Role? PLoS One: Vol.10, 9, e0137226.

Dzyadevycha SV, Arkhypovaa VN, Soldatkina AP, El'skayaa AV, Marteletb C and Jaffrezic-Renaultc N (2008). Amperometric Enzyme Biosensors: Past, Present and Future. Irbm: Vol.29, 2, 171-180.

Eggins BR (1996). Biosensors: An Introduction. John Wiley & Sons Ltd and BG Teubner. ISBN 978366305665-2, pp 211.

Eggins BR (2002). Chemical Sensors and Biosensors. John Wiley & Sons Ltd. ISBN: 0471 899143, pp 273.

Esclapez J, Zafrilla B, Martínez-Espinosa RM, Bonete MJ (2013). Cu-NirK from Haloferax mediterranei as an example of metalloprotein maturation and exportation via Tat system. Biochim Biophys Acta 1834: 1003-1009.

Glazier SA, Campbell ER, Campbell WH (1998). Construction and characterization of nitrate reductase-based amperometric electrode and nitrate assay of fertilizers and drinking water. Anal Chem 70: 1511-1515.

Inoue-Choi M, Jones RR, Anderson KE, Cantor KP, Cerhan JR, (2015). Nitrate and nitrite ingestion and risk of ovarian cancer among postmenopausal women in Iowa. Int J Cancer 137: 173-182.

Karube I, Suzuki M (1986). Novel immunosensors. Biosensors 2: 343-362.

Kross BC, Ayebo AD, Fuortes LJ (1992). Methemoglobinemia: nitrate toxicity in rural America. Am Fam Physician 46: 183-188.

Kuennen M, Jansen L, Gillum T, Granados J, (2015). Dietary nitrate reduces the O2 cost of desert marching but elevates the rise in core temperature. Eur J Appl Physiol 115: 2557-2569.

Larsen LH, Kjær T, Revsbech NP (1997). A Microscale NO(3)(-) Biosensor for Environmental Applications. Anal Chem 69: 3527-3531.

Larsen LH, Damgaard LR, Kjær T, Stenstrøm T, Lynggaard-Jensen A, Revsbech NP (2000). Fast Responding Biosensor for On-Line Determination of Nitrate/Nitrite in Activated Sludge. Water Research 34: 2463-2468.

Lledó B, Martínez-Espinosa RM, Marhuenda-Egea FC and Bonete MJ (2004). Respiratory Nitrate Reductase from Haloarchaeon Haloferax mediterranei: Biochemical and Genetic Analysis. Biochimica et Biophysica Acta (BBA)-General Subjects 1674: 50-59.

Marazuela D, Moreno-Bondi MC (2002). Fiber-optic biosensors--an overview. Anal Bioanal Chem 372: 664-682.

Martínez-Espinosa RM, Cole JA, Richardson DJ, Watmough NJ (2011). Enzymology and ecology of the nitrogen cycle. Biochem Soc Trans 39: 175-178.

Martínez-Espinosa RM, Richardson DJ, Butt JN, Bonete MJ (2006). Respiratory Nitrate and Nitrite Pathway in the Denitrifier Haloarchaeon Haloferax mediterranei. Biochemical Society Transactions 34: 115-117.

Martinez-Espinosa RM, Dridge EJ, Bonete MJ, Butt JN, Butler CS, (2007). Look on the positive side! The orientation, identification and bioenergetics of 'Archaeal' membrane-bound nitrate reductases. FEMS Microbiol Lett 276: 129-139.

Martínez-Espinosa RM, Richardson DJ, Bonete MJ (2015). Characterisation of chlorate reduction in the haloarchaeon Haloferax mediterranei. Biochim Biophys Acta 1850: 587-594.

Nájera-Fernández C, Zafrilla B, Bonete MJ, Martínez-Espinosa RM (2012). Role of the denitrifying Haloarchaea in the treatment of nitrite-brines. Int Microbiol 15: 111-119.

Nielsen M, Larsen LH, Jetten MS, Revsbech NP (2004). Bacterium-based NO2- biosensor for environmental applications. Appl Environ Microbiol 70: 6551-6558.

Nielsen M, Gieseke A, de Beer D, Revsbech NP (2009). Nitrate, Nitrite, and Nitrous Oxide Transformations in Sediments along a Salinity Gradient in the Weser Estuary. Aquatic Microbial Ecology 55: 39-52.

Plumere´ N, Henig J, Campbell WH (2012). Enzyme-Catalyzed O2 Removal System for Electrochemical Analysis under Ambient Air: Application in an Amperometric Nitrate Biosensor. Analytical Chemistry 84: 2141-2146.

Revsbech NP, Glud RN (2009). Biosensor for Laboratory and Lander-Based Analysis of Benthic Nitrate plus Nitrite Distribution in Marine Environments. Limnology and Oceanography: Methods 7: 761-770.

Rodriguez-Mozaz S, Lopez de Alda MJ, Barceló D (2006). Biosensors as useful tools for environmental analysis and monitoring. Anal Bioanal Chem 386: 1025-1041.

Saigal R, Goyal LK, Agrawal A, Wadhwani D, Mittal P, (2014). Methaemoglobinaemia as a result of nitrite poisoning. J Assoc Physicians India 62: 737-738.

Siontorou CG, Georgopoulos KN (2016). A Biosensor Platform for Soil Management: The Case of Nitrites. Journal of Cleaner Production 111: 133-142.

Slonczewski JL, Coker JA, DasSarma S (2010). Microbial Growth with Multiple Stressors. Microbe 5: 110-116.

Song P, Wu L, Guan W (2015). Dietary Nitrates, Nitrites, and Nitrosamines Intake and the Risk of Gastric Cancer: A Meta-Analysis. Nutrients 7: 9872-9895.

Sowjanya AP, Rao M, Vedantham H, Kalpana B, Poli UR, (2015). Correlation of Plasma Nitrite/Nitrate Levels and Inducible Nitric Oxide Gene Expression among Women with Cervical Abnormalities and Cancer. Nitric Oxide 52: 21-28.

Taylor CJ, Bain LA, Richardson DJ, Spiro S, Russell DA (2004). Construction of a whole-cell gene reporter for the fluorescent bioassay of nitrate. Anal Biochem 328: 60-66.

Wilkins ES (1989). Towards implantable glucose sensors: a review. J Biomed Eng 11: 354-361.

Zhao Z, Jiang H (2010). Enzyme-based Electrochemical Biosensors. In "Biosensors", Chapter 1. Book edited by Pier Andrea Serra, ISBN 978-953-7619-99-2.

ISSN : 0976-4550

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