Biosensors and Bioelectronics 94 (2017) 433–437

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

A novel versatile microbiosensor for local hydrogen detection by means of scanning photoelectrochemical microscopy

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ARTICLE INFO

Keywords:
Hydrogenase
Viologen-modified redox polymer
Oxygen-protection shield
SECM
Hydrogen biosensor
PS1-Pt

ABSTRACT

The development of a versatile microbiosensor for hydrogen detection is reported. Carbon-based microelectrodes were modified with a [NiFe]-hydrogenase embedded in a viologen-modified redox hydrogel for the fabrication of a sensitive hydrogen biosensor By integrating the microbiosensor in a scanning photoelectrochemical microscope, it was capable of serving simultaneously as local light source to initiate photo(bio) electrochemical reactions while acting as sensitive biosensor for the detection of hydrogen. A hydrogen evolution biocatalyst based on photosystem 1-platinum nanoparticle biocomplexes embedded into a specifically designed redox polymer was used as a model for proving the capability of the developed hydrogen biosensor for the detection of hydrogen upon localized illumination. The versatility and sensitivity of the proposed microbiosensor as probe tip allows simplification of the set-up used for the evaluation of complex electrochemical processes and the rapid investigation of local photoelectrocatalytic activity of biocatalysts towards light-induced hydrogen evolution.

1. Introduction

The global demand for energy sources that can satisfy the need of an ever-growing population and the adverse consequences for the environment originating from burning of fossil fuels have turned to the development of an economy using more efficient, clean and renewable energy sources. The increasing interest for a hydrogen-based economy and possible replacement of traditional fossil fuels requires the development of a sustainable hydrogen production. In this regard, particular interest is directed towards the search and development of catalysts or biocatalysts capable of performing hydrogen evolution from water (Krassen et al., 2011; Reisner, 2011; Vesborg et al., 2015; Zheng et al., 2014). Fast and reliable methods are required to assess the activity of (photo)catalysts for the hydrogen evolution reaction (HER).

Of particular interest in the evaluation of catalytic materials is the laterally-resolved characterization of a surface in search for local active hot-spots for catalysis. This allows the study of heterogeneous samples and materials libraries in search of a highly active material with a particular composition. Scanning electrochemical microscopy (SECM) allows studying the local electrochemical or electrocatalytic properties of a surface. By recording the current obtained at an accurately positioned SECM tip electrode as function of tip location, chemical images are obtained, which translate to differences in reaction rates at different locations at the surface. SECM has found wide application in different fields, among them, the measurements of local heterogeneous reaction kinetics, homogeneous kinetics and the investigation of bioctalytic systems (Amemiya et al., 2008; Edwards et al., 2006; Sun et al., 2007). The further demand for exploring new materials and complex catalytically active surfaces requires the development of new methodologies on the basis of SECM principles (Bertoncello, 2010; Cannan et al., 2011; Zoski, 2015). Local detection of hydrogen evolution using SECM has been reported for the evaluation of the electrocatalytic activity of electrocatalysts (Jamali et al., 2015; Jedraszko et al., 2014) or the corrosion of metals (Tefashe et al., 2014). Further improvement was made recently by implementing a

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http://dx.doi.org/10.1016/j.bios.2017.03.037
Received 16 December 2016; Received in revised form 15 March 2017; Accepted 17 March 2017
Available online 18 March 2017
0956-5663/

scanning photoelectrochemical microscopy (SPECM) set-up that allows high-resolution visualization of the photoactivity of semiconductors as well as photoactive protein complexes such as photosystem 1 (PS1) and photosystem 2 (PS2) under controlled and localized irradiation conditions (Zhao et al., 2015; Conzuelo et al., 2017). A Pt microelectrode was used for the collection of biophotocatalytically evolved hydrogen which showed limited sensitivity when smaller electrode tips are used for the high resolution analysis of a given surface. In addition, the use of unmodified electrode surfaces and the necessary relatively high applied potential for hydrogen detection make this approach prone to the influence of interfering compounds.

Enzyme-based biosensors play an important role in the determination of a number of molecules of interest due to their inherent sensitivity and especially high selectivity. Moreover, their unique features such as high efficiency at mild pH values and temperature conditions, rapid response and capability of miniaturization make them an attractive tool for the analysis and continuous monitoring of a variety of different analytes (Sassolas et al., 2012). Hydrogenases (H_2ases) are nature's efficient biocatalysts for the reversible $H_2/2H^+$ interconversion at high turnover rates and low overpotentials. However, the application of hydrogenases in biosensors has been limited so far, since the enzymes suffer from deactivation by both oxygen and high applied potentials (Lubitz et al., 2014). Recently, we have proposed an elegant method to overcome these limitations and to shield hydrogenases from both deactivation by molecular oxygen as well as high applied potentials (Plumeré et al., 2014). By integrating a [NiFe]-hydrogenase within a viologen-based redox hydrogel, an efficient bioelectrode for H_2 oxidation was achieved even in the presence of high concentrations of oxygen. Further work evaluated the protective role of the viologen-based redox polymer from oxygen and high potentials (for a detailed description, the reader is referred to: Fourmond et al., 2015) and allowed the successful protection of the enzyme when replacing a [NiFe]-hydrogenase by an [FeFe]-hydrogenase which is usually irreversibly deactivated by oxygen (Oughli et al., 2015). These results open up a new possibility for the development of hydrogenase-based hydrogen biosensors.

We present a hydrogenase/viologen-based redox hydrogel modified microelectrode integrated into a scanning photoelectrochemical microscopy (SPECM) set-up using the hydrogen biosensor simultaneously as light source for local illumination as well as for the localized determination of photocatalytically evolved hydrogen. To demonstrate the ability of the developed hydrogen microbiosensor to locally detect (bio) photocatalytically generated hydrogen, a gold surface modified with a composite of photosystem 1/Pt nanoparticle biocomplexes (PS1-Pt) embedded in an Os-complex modified redox polymer was selected (Zhao et al., 2015). By polarizing the PS1-Pt/redox polymer modified Au surface at a suitable potential for light-induced hydrogen evolution, variations in the hydrogen production rate were successfully visualized by scanning the developed hydrogen microbiosensor across the sample in the sample generation/tip collection mode of SECM.

2. Materials and methods

2.1. Reagents and chemicals

Citric acid monohydrate, sodium phosphate and potassium chloride were from J.T. Baker. Tris(hydroxymethyl)aminomethane-HCl (Tris–HCl) was from AppliChem. Poly(ethyleneglycol)diglycidyl ether (PEGDGE) was from Polysciences. Hexaammineruthenium (III) chloride was from Sigma-Aldrich. All chemicals were of analytical grade and used without further purification. All solutions were prepared using deionized water (ρ=18 MΩ cm). The viologen-functionalized polyethylenimine polymer was synthesized as described elsewhere (Plumeré et al., 2014). [NiFe]-hydrogenase from *Desulfovibrio vulgaris* Miyazaki F (*Dv*MF [NiFe]-H_2ase) was obtained and purified as described previously (Fichtner et al., 2006; Yagi et al., 1976). The poly(1-vinylimidazole-*co*-allylamine) polymer modified with $[Os(bpy)_2Cl]^+$ was synthesized as described earlier (Badura et al., 2008; Sokol et al., 2016). PS1-Pt bioconjugates were prepared as described in (Zhao et al., 2015). Briefly, freshly synthesized mercaptosuccinic acid-stabilized Pt nanoparticles were coupled to PS1 extracted from *Thermosyne chococcus elongatus* (Badura et al., 2011) by incubating overnight in the dark at 4 °C. The self-assembled PS1-Pt complexes were purified by microfiltration in order to remove unbound Pt nanoparticles.

2.2. Preparation of hydrogenase-modified microelectrodes

Carbon microelectrodes were prepared using a modified procedure for the preparation of carbon nanoelectrodes (Clausmeyer et al., 2014). Quartz capillaries (Sutter Instruments; outer diameter 1.2 mm, inner diameter 0.9 mm) were pulled using a P-2000 laser puller (Sutter Instruments) to obtain fine tips. A dense layer of carbon was deposited on the inner wall of the pulled capillaries by pyrolysis of a butane/propane (80/20) mixture heated under inert atmosphere (Ar). The tips were then polished on polishing paper of different grain sizes until an opening of desired dimensions was obtained. Then, the tip of the prepared microelectrode was sealed with carbon paste (Leit-C, Plano), removing the excess deposited on the sheath of the capillary. A copper wire coated with silver cement (Leitsilber, Plano) was inserted into the opposite end of the capillary for electrical connection. The prepared carbon microelectrodes were carefully polished and characterized by recording cyclic voltammograms in 5 mM $[Ru(NH_3)_6]Cl_3$ containing 0.1 M KCl. A typical steady-state cyclic voltammogram for disk-shaped microelectrodes was obtained with calculated dimensions of 18–20 µm in diameter. These carbon microelectrodes were used as basis for the fabrication of hydrogenase-based microbiosensors. For this, the tip of the carbon microelectrode was inserted into a mixture of *Dv*MF [NiFe]-hydrogenase (35 µM) and the viologen-based polymer (10 µg $µL^{-1}$) and incubated overnight at 4 °C. The obtained modified microelectrode is schematically depicted in Fig. 1. Carbon fiber electrodes prepared as described elsewhere (Schulte and Chow, 1996) were also used as electrodes for the fabrication of similar hydrogen microbiosensors for comparison.

2.3. Cyclic voltammetry

Cyclic voltammograms were recorded with an Autolab PGSTAT30 potentiostat (Metrohm-Autolab) using a three-electrode system comprising the hydrogen biosensor as working electrode, a miniaturized Ag/AgCl/3 M KCl as reference electrode and a Pt wire as counter electrode. All potentials are referred to the Ag/AgCl/3 M KCl reference electrode.

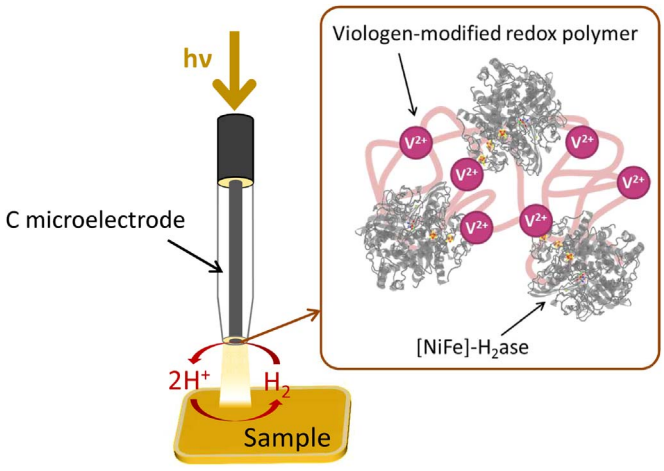

Fig. 1. Schematic representation of the [NiFe]-hydrogenase/viologen-based polymer modified hydrogen microbiosensor used as SPECM tip.