

Enzyme/semiconductor nanoclusters combined systems for novel amperometric biosensors

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Abstract

In this work quantum-sized CdS nanocrystals were synthesized using a quaternary water-in-oil microemulsion and immobilized onto gold working electrode by self-assembled monolayers techniques. Formaldehyde dehydrogenase was covalently immobilized onto a protecting membrane, which was stratified on part of the semiconductor nanoparticles modified electrode. The covalent enzyme immobilization has been required to improve the stability of the catalytic oxidation of formaldehyde, which occurs after light stimulation of the semiconductor through the electron/hole recombination. A study about the best electrochemical oxidation potentials under different flow conditions was performed. Preliminary sensor stability and interferences tests were also carried out, for a sensitive and selective detection of formaldehyde. A detection limit of 41 ppb of formaldehyde was calculated and an operational stability of 6 h was achieved under flow conditions by means of this novel amperometric biosensor based on FDH-semiconductor hybrid systems, not requiring NAD⁺/NADH as charge transfer in the enzymatic reaction.

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1. Introduction

Nanostructured materials have proven as one of the most powerful tool in new trend of technology and research, due to their absolutely peculiar properties at nanometer size scale. Many studies have shown that optical, mechanical, photocatalytic and transport properties drastically changes, depending on quantum size effect, as the mean diameter of the particles is in the exciton size regime (i.e. <10 nm) [1–9]. Nanosized semiconductors find large applications in lumines-

cence, non-linear optic, catalysis, optoelectronics and photochemistry [10–13].

New combined structures made of semiconductor NCs and enzymes have been already designed. In such systems electron and hole generated respectively in the conduction and valence bands can be used for the substrate reduction (oxidation) through electron acceptor (donor). The electronic transfer mechanism of hole and electron towards the interface enzyme/NCs depends strongly upon the redox potential of semiconductor, the enzymatic reaction and the presence of a substance, which can be reversibly oxidized and reduced.

Nanoparticles architectures on electrode supports pushed towards the development of electronic nano-devices such as in metal/semiconductor arrays assembled on electrodes, multi enzymatic arrays or biochips [14–17]. Extensive research has been also devoted to the surface activation, by organic monolayers, chemicophysical modification or

Abbreviations: CTAB, hexadecyl trimethyl ammonium bromide; FDH, formaldehyde dehydrogenase; GA, glutaraldehyde; LOD, limit of detection; NAD, nicotinamide adenine dinucleotide; NADH, nicotinamide adenine dinucleotide (reduced form); NCs, nanoclusters; PB, phosphate buffer 0.1 M (pH = 8.0); POH, pentanol; RE, reference electrode (Ag/AgCl); SAMs, self-assembled monolayers; WE, working electrode; w/o, water-in-oil

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biomaterials immobilization procedure [18–21]. The concept of self-assembled monolayers has been extended to insert metal and/or nanosized semiconductors onto several surfaces [22–30].

Formaldehyde is a commercially widespread chemical, largely produced for domestic, industrial and clinical uses, which is naturally present in fruits, vegetables, flesh, fishes, biological fluids. Formaldehyde detection became an environmental and clinical issue, because of its toxicological effect for consumers and proved mutagenic effects on several living organisms [31,32]. Most of the main established analytical methods for formaldehyde detection, including visible absorption [33], liquid chromatography (HPLC, IC) [34–36], gas chromatography [37–38] and fluorimetry [39] reached high sensitivity but basically they require toxic reagent and skilled personnel, especially for the sample treatment, resulting in impractical real-time measurements. Recent efforts have turned towards the development of rapid and specific electrochemical biosensors based on NADH-dependent dehydrogenase, using different mediators for NADH electro-oxidation either in batch reactors or under flow conditions [40–47].

Unfortunately, the NAD^+/NADH couple generally exhibits hard electrochemical oxidation of NADH. The most redox NAD^+/NADH dependent enzymes reveal fundamental difficulties, due to their poor stability, high costs and electrical contacting with electrode. Hence, semiconductor nanocrystals represent a possible route to enhance the electron/hole transfer efficiency, overcoming the problems due to the NAD^+/NADH dependence.

We attempted to use CdS nanosized semiconductors as new charge transfer agents in cofactor-dependent enzyme reactions, in order to construct novel amperometric biosensors for online formaldehyde monitoring. Nanosized semiconductors coupled to formaldehyde dehydrogenase (FDH) enzyme resulted in a hybrid system able to achieve the electron transport towards or from the electrode surface after suitable photo-activation. Curri et al. [48] already presented an enzyme-semiconductor hybrid, where the FDH enzymatic behavior was compared in water and micellar solution, investigating the enzymatic activity in the presence of semiconductor nanoparticles. Kinetic measurements carried out in microemulsive systems confirmed the enzymatic activity is fully retained in different ambient, especially in quaternary microemulsion, where a sufficient variety of (II–VI) semiconductors can be easily synthesized, without resulting in a denaturation of the enzyme properties. The successful enzymatic oxidation of formaldehyde under illumination was also demonstrated in FDH-semiconductor hybrid, as well as the reversibility of the reaction [48]. On the other hand, the low stability of the electrochemical response after each sequence of illumination still remains a limitation, owing to an observable photo-corrosion effect and to the possible inhibition of the enzymatic redox site.

Our purpose was to develop the presented hybrid system for more stable formaldehyde detection, without using

the NAD^+/NADH couple for the charge transfer. Covalent immobilization of FDH on protecting functionalized membranes combined with semiconductor-based electrode was required to avoid the enzyme denaturation in solution, especially when using flow conditions. The influence of other redox substances was also investigated. The sensitivity of this novel system cannot be considered as practically comparable with respect to previous NAD-dependent biosensors for formaldehyde detection.

2. Experimental

2.1. Chemicals and reagents

Formaldehyde dehydrogenase (FDH) from *Pseudomonas putida* (EC 1.2.1.46, with specific activity between 4 and 6 units per mg), formaldehyde (stock solution: 4% w/v), glucose, glycine, GA (25% aqueous solution, solid), paracetamol, methanol, L(+)-ascorbic acid, sodium sulphite salt and cysteine were purchased from Sigma. Pre-activated Nylon ID membranes (ImmunodyneTM) were purchased from Pall Italia (Milan, Italy) and used according to the manufacturer's instructions. Buffer solution was prepared immediately before using. Cetyl-trimethyl ammonium bromide (CTAB) surfactant, pentanol, hexane, hexanedithiol, cadmium nitrate, sodium sulphide, potassium and sodium phosphate salts were from Merck, all other chemical were used without further purification and were of analytical grade. All reagents and electrolyte solution were prepared using twice distilled water.

2.2. Nanocrystals synthesis and immobilization on gold

Enormous improvements has been made in preparing and characterizing nanosized semiconductors of II–VI, III–V and IV type, metal doped or undoped, by means of host–guest inclusion chemistry or colloidal synthesis [49,50]. New synthetic methods are based on thermal decomposition of precursors in coordinating solvents and capping agents at relatively high temperature [51–55]. Nanostructured CdS preparation in quaternary water-in-oil microemulsive systems has been showed as one of the most simple and successful synthetic route, as extensively reported in several papers [56–61], to regulate at will the semiconductor particle size and properties, due to the simplicity of the synthesis and the well defined properties of the reverse micelles. In this paper CdS nanoparticles were prepared using a quaternary w/o microemulsion, formed by CTAB, as cationic surfactant, pentanol (the co-surfactant), *n*-hexane and water, mixing equivalent amounts of precursor salts in micellar solution, i.e. cadmium nitrate and sodium sulphide. The nanocrystals size were modulated and controlled by varying respectively water to surfactant ($W_0 = \text{H}_2\text{O}/\text{CTAB}$) and pentanol to surfactant ($P_0 = \text{POH}/\text{CTAB}$) ratios. In fixed experimental conditions (CTAB at 0.1 M, pentanol content in the range between 8

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