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New mediators for biosensors based on PQQ-dependent alcohol dehydrogenases

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ABSTRACT

A set of 10 quinoidal compounds synthesized enzymatically using laccase from *Coriolopsis byrsina* GRB13 have been examined as redox mediators applicable for bioelectrocatalytic systems using two types of pyrroloquinoline quinone-dependent alcohol dehydrogenases – periplasmic (s-PQQ-ADH) and membrane-bound (m-PQQ-ADH). The efficiency of the biosensors have been evaluated for both types of enzymes and varied in range from 2×10^{-7} A for Phenazine-2,3-diamine to 26×10^{-7} A for 2-(3-Nitroanilino)-1,4-benzoquinone. Experimental data correlated with the molecular properties of mediators using results of *ab initio* quantum chemical calculations. The oxidation potential of mediators as well as the maximum current (I_{max}) of biosensors correlated with the calculated energy of the lowest unoccupied molecular orbital (E_{LUMO}) of mediators. In the case of I_{max}, this result indicates the rate-limiting character of enzymatic mediator reduction in the overall process of biosensor response. The proposed approach demonstrates that the advance determination of molecular properties of potential mediators by *ab initio* quantum chemical calculations might be a useful instrument in the development process of the redox enzymes based biosensors.

1. Introduction

Owning to the enormous possibilities for the engineering of electrochemical biosensors with desirable properties, understanding the principles of bioelectrocatalysis becomes essential. Bioelectrocatalysis is an acceleration of the electrochemical reaction by using biological catalysts which usually are enzymes or whole cell systems [1]. The integration of them into industrial processes such as biosensors design or for effecting the conversion of readily available, inexpensive starting materials to high value products is strongly demanded nowadays. Enzymes are fully recyclable catalytic proteins that frequently display exquisite chemo-, enantio- and regio-selectivity and operate under mild conditions of pH and temperature. These characteristics make them cost-effective and sustainable catalysts for a wide range of chemical transformations. The exquisite selective and reactive properties of biocatalysts provide an effective synthetic strategy for creating optically pure molecules, which are essential for the manufacture of many pharmaceuticals, agrochemicals, materials and food ingredients [2]. In recent years, chemists turned their attention to the application of biocatalysis in the synthesis of chiral chemicals using either enzymes or whole cells [3]. Many methods have been developed and are currently used to prepare chiral products, such as chemical asymmetric synthesis, catalytic kinetic resolution and chiral chromatography, etc. [4,5,6,7].

The use of different classes of enzymes for the catalysis of many different types of chemical reactions is capable of generating a wide variety of chiral compounds [8]. One of them – alcohol dehydrogenases (ADH) could be used in the synthesis of chiral aldehydes. ADH is widely distributed in many different types of organisms ranging from bacteria to mammals. It is usually NAD(P)-dependent enzymes present in the cytoplasm. Though in order to use these enzymes in biocatalysis, it is first necessary to establish an effective cofactor regeneration system. The high costs of pyridine nucleotide cofactors have limited the applications of NAD(P)-dependent oxidoreductases on an industrial scale. Although NAD(P)H regeneration systems have been widely studied, $NAD(P)^+$ regeneration, which is required in reactions where the oxidized form of the cofactor is used, has been less explored, particularly in whole-cell biocatalytic processes. In contrast, PQQ-dependent ADHs are rather unique and are found in only a narrow range of bacteria species, and are localized only in the periplasmic fraction [9]. While various oxidoreductases are used for bioelectrocatalysis, however, due to insensitivity to oxygen and tightly bound cofactor, PQQ-dependent enzymes have clear advantages

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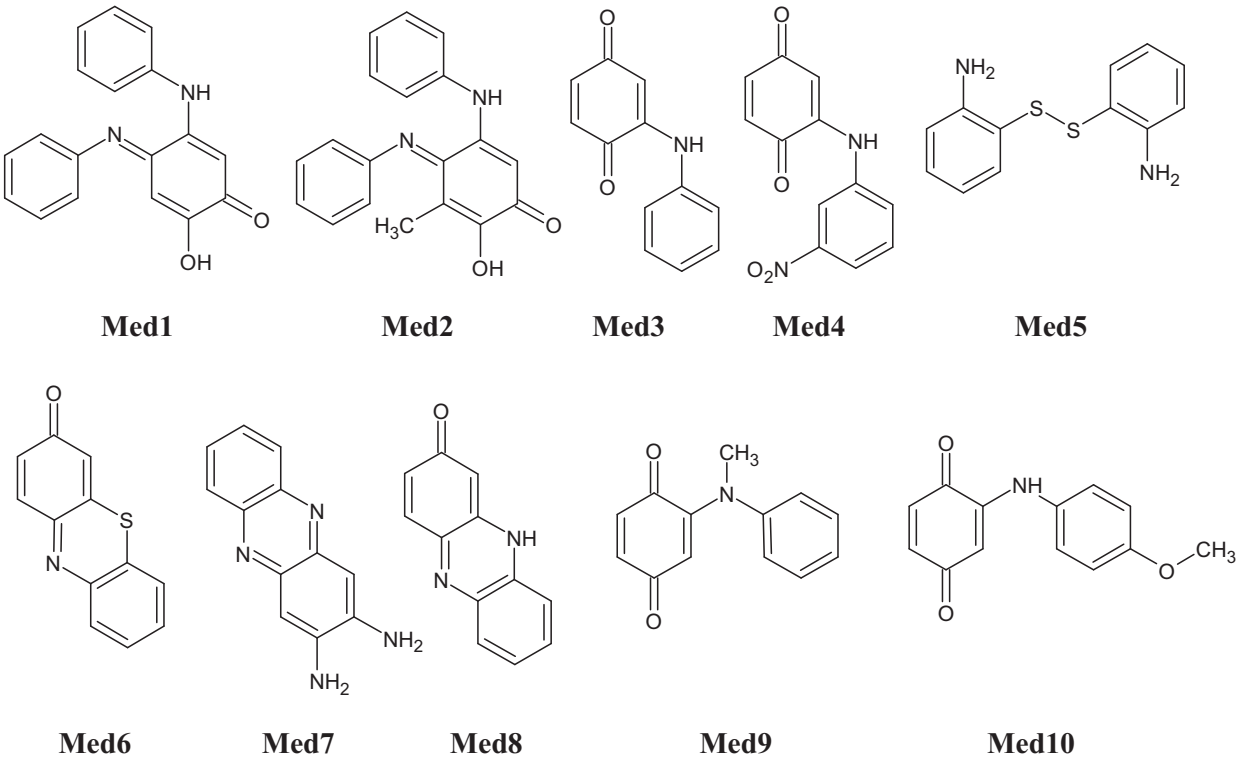

Fig. 1. Chemical structures of synthesized mediators.

when comes to development of various electrocatalytic processes [10].

PQQ-dependent ADHs are very promising tools when applied to the various designs of amperometrical biosensors [11] or biofuel cells [12]. The PQQ dependent enzymes can be as alternative biocatalysts for wide range of reactions. The implementation of industrially promising biocatalysts, especially oxidoreductases, is confronted with difficulties concerning availability of an efficient redox mediator, which promotes regeneration of cofactors by improving electron transfer between active site of enzyme and electrode surface. A great number of electron donors or acceptors are known and used in chemical technologies, however many of them suffer from low efficiency or are expensive for application in a bulk synthesis. Thus, there still is lack of cheap but efficient redox mediators applicable for enzyme catalyzed redox reactions. Recently laccase from *Coriolopsis byrsina* GRB13 was isolated and enzyme catalyzed synthesis of various substituted quinones and hydroquinones was shown [13]. Two synthesized quinones (2-(N-methylanilino)-1,4-benzoquinone and 2-(3-nitroanilino)-1,4-benzoquinone) were powerful redox mediators applicable for bioelectrocatalytic systems based on PQQ-dependent glucose dehydrogenase. In this work a set of 10 compounds have been synthesized enzymatically by L. Marcinkeviciene and colleagues as proposed in the protocol [13] and tested as redox mediators applicable for PQQ-dependent alcohol dehydrogenases (PQQ-ADH). Moreover, two structurally different types of pyrroloquinoline quinone-dependent alcohol dehydrogenases – periplasmic (s-PQQ-ADH) and membrane-bound, (m-PQQ-ADH) have been examined. Aiming to discover all essentials for effectiveness of the redox mediators applied in bioelectrocatalytic systems using PQQ-ADHs in parallel with experimental approach, quantum chemical calculations have been applied for the evaluation of molecular properties (structure, energy, etc.).

2. Materials and methods

2.1. Materials

m-PQQ-ADH (specific activity 38.1 U/mg) was purified from *Gluconobacter* sp. 33 as described [14], and was used as a solution in 20 mM potassium phosphate buffer (pH 7.0) containing 0.02% Triton X-100 and 0.5% sucrose.

s-PQQ–ADH (specific activity 2 U/mg) was purified from *Pseudomonas putida* HK5 and was used as a solution in 5 mM Tris buffer solution (pH 8.5), containing 5 mM Ca^{2+}. The enzymes substrates were used as the acetate buffer solutions, pH 6.0 containing 100 mM of 1.2-propandiol or 100 mM of ethanol.

Sodium acetate, acetic acid, $CaCl_2$ and 1.2-propandiol were obtained from J.T. Baker (Holland, NL). 95–97% H_2SO_4, Penta, US. Ethanol, KCl were purchased from Riedel-de Haen (DE). Amorphous carbon Raven M was purchased from Columbian Chemicals Co (Atlanta, US). H_2O_2 (ChemPur, DE).

2.2. General procedure for the synthesis of aminated quinones

Laccase-catalyzed reaction was performed at room temperature in 20 mM sodium acetate buffer pH 5.0 according to the protocol [13]. Four millimolar solution of catechols or hydroquinone in 100 mL buffer was oxidized with 50 μL laccase (activity 1.2 U/μL) for 2–3 h. When oxidation was completed according TLC and UV/VIS spectrum, an equimolar amount of the appropriate amine [13, Table 3] was added. Thin-layer chromatography was carried out on 25 TLC aluminium sheets coated with silica gel 60 F_{254} (Merck). Products were isolated approximately 20 h after addition of amine. Products with low solubility in buffer were collected by centrifugation (4000 g, 20 min), soluble products were purified by column chromatography (silica gel 60 (0.04–0.0630 nm) (Merck), eluent hexane/ethyl acetate). The structure and the purity of the