Some quinone derivatives as redox mediators for PQQ-dependent glucose dehydrogenase

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Some quinone derivatives (2,3-dichloro-1,4-naphthoquinone, 2-methyl-6-methoxy-1,4-benzoquinone, 1,4-naphthoquinone, 2-hydroxy-1,4-naphthoquinone, trimethyl-1,4-benzoquinone, tetramethyl-1,4-benzoquinone, 9,10-phenanthrenequinone, 2-methyl-1,4-naphthoquinone) were investigated as a redox mediators for pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (GDH) purified from *Erwinia* sp. *34-1*. Amperometrical response to glucose addition in the sample containing GDH and quinone was investigated. The results show that 2-methyl-6-methoxy-1,4-benzoquinone, 1,4-naphthoquinone, trimethyl-1,4-benzoquinone are the best redox mediators for GDH.

Key words: quinones, glucose determination, quinoprotein, glucose dehydrogenase

INTRODUCTION

Quinoproteins are a relatively new class of flavin- and NADH-independent oxidoreductases that contain the *ortho*-quinoind cofactor PQQ. They can be successfully applied in various analytical systems for detection of biologically active analytes. Since PQQ-dependent enzymes are oxygen-independent, they are of especial interest during creation of oxygen-independent amperometrical biosensors [1]. One of the most important problems in medicine and food control is determination of glucose. Some of PQQ-dependent enzymes like PQQ(heme)-dependent alcohol dehydrogenase are able to transfer electrons directly to carbon surface [2] or polypyrrole backbone [3]. However, PQQ-dependent glucose dehydrogenase is unable to transfer electrons directly to solid conducting surfaces or backbone of π-conjugated polymers. That is the reason why development of suitable redox mediators is of especial interest. Since the present time some soluble redox mediators (*e.g.*, PMS [4]) were applied in the design of amperometrical analytical systems, but these mediators are useful only for single-use biosensors. For creation of biosensors for continued measurements, insoluble mediators are much more promising. For this purpose, carbon electrodes modified with water-insoluble organic electron shuttles (*e.g.*, 7,7,8,8-tetracyanoquinodimethane [4]) can be applied in the design of biosensors. Entrapment of GDH within a matrix based on polypyrrole modified with covalently bound osmium complexes [5] can be exploited as a more successful approach to create biosensors for continued measurements. However, in this case the most important problem is the complicated copolymerization conditions. The electrons from the reduced form of the enzyme can be also transferred via the polyquinonic polymers attached to the GDH. The enzyme modified in this way has been used in reagentless glucose biosensors [6], however, the responses of such biosensors are not high enough and the activity of GDH is significantly reduced, since attachment of polyquinonic compounds requires manipulations with strong oxidants. All the mentioned reasons forced us to search for new redoxable compounds suitable to transfer electrons to the active site of GDH. The aim of this work was to investigate some quinone compounds as potential redox mediators for GDH.

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MATERIALS AND METHODS

Reagents. GDH from *Erwinia* sp. 34-1 (specific activity 17–18 U/mg of protein) was isolated and purified at the Institute of Biochemistry (Vilnius, Lithuania) by the method published earlier [7]. Carbon rod ultra F electrodes (cat. No. 001281-10) 3 mm in diameter were purchased from Ultra Carbon Division (Bay City, USA). Glucose was obtained from Ceretor (Germany) and dimethylsulfoxide from Reakhim (Kiev, Ukraine). 2,3-Dichloro-1,4-naphthoquinone (1), 2-hydroxy-1,4-naphthoquinone (4), tetramethyl-1,4-benzoquinone (6), 2-methyl-1,4-naphthoquinone (8) (Sigma, St Louis, MO) were used as received. 2-Methyl-6-methoxy-1,4-benzoquinone (2) and trimethyl-1,4-benzoquinone (5) were synthesized according to the methods described previously [8]. Trimethyl-1,4-benzoquinone (5) was synthesized from trimethyl-1,4-hydroquinone Aldrich, (Steinheim, Germany) according to a previously described protocol [8]. 1,4-Naphthoquinone (3), 9,10-phenanthrenequinone (7) were obtained from Reakhim (Shostkino, Russia), purified by sublimation under vacuum or recrystallized from benzene or ethanol. 100 mM solutions of quinones were prepared in dimethylsulfoxide. All aqueous solutions were prepared by using HPLC grade water purified in a Purator-B Glass Keramik (Berlin, Germany). All experiments were carried out in 0.01 M potassium phosphate buffer (pH 7.0) containing 0.1 M potassium chloride and 1 mM Trilone-B obtained from Analita (Lithuania).

Equipment. All amperometric measurements were performed with a PA-2 polarograph Laboratorny Pristroje (Czech Republic) connected to XY recorder (Schlotheim, Germany) and a three-electrode cell consisting of a working carbon electrode, a saturated Ag/AgCl reference electrode and a Pt auxiliary electrode.

Electrochemical investigations. Cyclic voltammetry of quinone compounds was performed in a solution (200 µM) of a corresponding quinone in a 1:4 mixture of dimethylsulfoxyde and 0.01 M phosphate buffer (pH 7.0) with 0.1 M KCl. Steady-state current responses at 0.1 V *vs.* the Ag/AgCl reference electrode to glucose addition were measured in a 1 ml electrochemical cell containing 0.01 M potassium phosphate buffer (pH 7.0), 0.1 M KCl and 0.5 U/ml PQQ-GDH. At least 5 min before the enzymatic reaction was started by addition of glucose (10 µl), a solution (10 mM) of corresponding quinones was added into the electrochemical cell.

RESULTS AND DISCUSSION

Cyclic voltammograms of all quinone compounds showed a reversible character, indicating that theo-retically all these compounds can be applied as electron transfer mediators in electrochemical systems. The redox potentials determined by cyclic voltammetry are presented in Table. The results obtained are in agreement with those reported by other authors [9].

Table. **Electrochemical data on the quinines studied**

Quinones	$E_{1/2}$, V	Sensitivity, nA/mM
2,3-Dichloro-1,4-naphthoquinone	−0.02	132
2-Methyl-6-methoxy-1,4-benzoquinone	−0.10	2346
1,4-Naphthoquinone	−0.17	2319
2-Hydroxy-1,4-naphthoquinone	−0.38	–
Trimethyl-1,4-benzoquinone	−0.01	2550
Tetramethyl-1,4-benzoquinone	−0.20	–
9,10-Phenanthrenequinone	−0.24	103
2-Methyl-1,4-naphthoquinone	−0.26	–

The steady-state amperometric responses were investigated by increasing glucose concentrations in the reaction cell containing GDH and a corresponding quinone. In the case of 2-hydroxy-1,4-naphthoquinone (4), tetramethyl-1,4-benzoquinone (6), 2-methyl-1,4-naphthoquinone (8) no noticeable amperometric response was detected. In the presence of 2,3-dichloro-1,4-naphthoquinone (1) and 9,10-phenanthrenequinone (7) the steady-state responses were in the range of 95 nA/mM and 50 nA/mM, respectively. The highest steady-state responses were obtained when 2-methyl-6-methoxy-1,4-benzoquinone (2), 1,4-naphthoquinone (3) and trimethyl-1,4-benzoquinone (5) were used as redox mediators. Response of the electrode was in the range of 1.6–1.8 mA/mM (Figure).
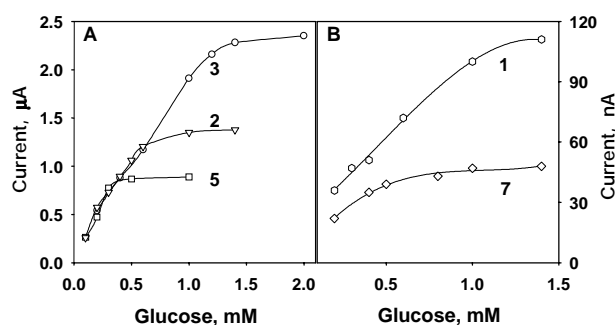

Figure. A. Steady-state current response to step-by-step addition of glucose in the presence of 2-methyl-6-methoxy-1,4-benzoquinone (2), 1,4-naphthoquinone (3), trimethyl-1,4-benzoquinone (5);

B. Steady-state current response to step-by-step addition of glucose in the presence of 2,3-dichloro-1,4-naphthoquinone (1), 9,10-phenanthrenequinone (7). At + 0.1 V *vs.* Ag/AgCl, in 0.01 M potassium phosphate buffer (pH 7.0) containing 0.5 U/ml of GDH

Comments and future developments. Investigations of the steady-state currents showed no clear correlation between the obtained responses and the structure or $E_{1/2}$ of quinones studied during this work. The reaction rates of quinones with GDH will be investigated in the next study.

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CHINONINIØ JUNGINIØ KAIP REDUKCIJOS MEDIATORIØ NUO PQQ PRIKLAUSOMAI GLIUKOZËS DEHIDROGENAZEI PANAUDOJIMAS

S a n t r a u k a

Tirta galimybë panaudoti chinoninius junginius (2,3-dichlor-1,4-naftochinonà, 2-metoksi-6-metil-1,4-benzochinonà, 1,4-naftochinonà, 2-hidroksi-1,4-naftochinonà, trimetil-1,4-benzochinonà, tetrametil-1,4-benzochinonà, 9,10-fenantrachinonà, 2-metil-1,4-naftochinonà) kaip redukcijos mediatorius nuo pirolochinolino chinono (PQQ) priklausomai gliukozës dehidrogenazei (GDH), iðskirtai ið *Erwinia* sp. *34-1* kamieno. Buvo iðmatuota srovës pokyèio priklausomybë nuo gliukozës koncentracijos 0,5 U GDH tirpaluose su tiriamu chinoniniu junginiu. Nustatyta, kad ið ðiame darbe tirtø chinoniniø junginiø geriausi redukcijos tarpininkai gliukozës dehidrogenazei yra 2-metoksi-6-metil-1,4-benzochinonas, 1,4-naftochinonas, trimetil-1,4-benzochinonas.