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Sensitive amperometric detection of riboflavin with a whole-cell electrochemical sensor

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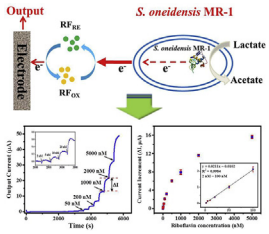
HIGHLIGHTS

- Riboflavin cycling system based on *Shewanella oneidensis* and its outwards electron transfer was investigated.
- An amperometric biosensor with *Shewanella* endogenous electron was developed for riboflavin detection.
- A low LOD (0.85 ± 0.09 nM) and high sensitivity (0.0211 ± 0.0023 μA/nM) was achieved.

GRAPHICAL ABSTRACT



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ABSTRACT

A novel whole-cell electrochemical sensor was developed and applied for sensitive amperometric detection of riboflavin. In this work, a whole-cell based riboflavin redox cycling system was characterized, in which electroactive bacteria *Shewanella oneidensis* MR-1 was employed as the biocatalyst to regenerate the reduced riboflavin after the electrode oxidation. This redox cycling system efficiently enhanced the electrochemical response of riboflavin and enabled a stable current output at poised electrode potential. Thus, a sensitive amperometric biosensing system for riboflavin detection was developed by integrating this whole-cell redox cycling system with the conventional riboflavin electrochemical sensor. Remarkably, this riboflavin biosensor exhibited high sensitivity (LOD = 0.85 ± 0.09 nM, *S/N* = 3), excellent selectivity and stability. Additionally, reliable analysis of real samples (food and pharmaceutical samples) by this biosensor was achieved. This work provided sensitive and practical tool for riboflavin detection, and demonstrated that the integration of electroactive bacteria and using its outwards electron transfer for redox cycling would be a powerful and promising strategy to improve the performance of electrochemical sensing system.

1. Introduction

Riboflavin (also known as Vitamin b_2) is a well-known water soluble vitamin and is essential to the human health. It is the central module for coenzymes as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which play vital roles in series of carbohydrates, fats and protein metabolism [1]. Deficiency of riboflavin would result in various metabolic disorder and diseases like stomatitis and skin rashes [2]. Meanwhile, the excess riboflavin is also threatening to human health since it is an efficient photosensitizer and would induce oxidative damage to tissues and DNA when exposed to UV [3,4]. More importantly, riboflavin cannot be produced in the human body, and has to be taken in from dietary source, supplements or medicines [2]. Thus, developing new

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strategies and techniques for efficient determination of riboflavin concentration is significative in forages, food, supplements quality control and clinical diagnoses.

Although most riboflavin detection techniques are depended on its distinctive fluorescence characteristic [5–8], the excellent redox property of riboflavin makes it applicable for electrochemical detection [9]. As a result, various electrochemical techniques have been developed for riboflavin determination [10–16]. Most of these riboflavin electrochemical sensors were based on the conventional voltammetry analyses including differential pulse voltammetry (DPV) [10,11], cyclic voltammetry (CV) [12,16] and square wave voltammetry (SWV) [17,18], in which the response current variation upon the electrode potential change is recorded. Despite the subtle differences on the electrode potential control mode, these voltammetry sensors are principally based on direct riboflavin transformation at electrode surface upon consecutive electrode potential change. As a result, most of these reported attempts were focused on electrode modification [11,14,16,19,20].

However, further improvement of the riboflavin detection performance through electrode modification is difficult. Firstly, the margin effect of electrode modification gradually becomes weak since riboflavin originally has excellent electrochemical activity and fast electron transfer rate [21,22]. Secondly, the output signal in conventional riboflavin electrochemical sensor is limited by the local riboflavin concentration as it is irreversibly consumed during electrochemical measurement. As a result, new strategies should be developed and integrated into conventional riboflavin electrochemical sensor in order to improve its analytical performance [10,13,23].

On the other hand, coupling proper reactions to regenerate riboflavin after it is registered at electrode (via oxidation/reduction reaction) may lead to repeated response of single riboflavin molecule and enhance current output of respective riboflavin electrochemical sensor. The idea of developing such redox cycling system for signal amplification attracts vast research interests and accordingly a wide range of ultra-sensitive detections of diagnostic biomarkers, pathogens, toxic heavy metal ions and pesticides have been demonstrated [24–28]. Meanwhile, most of these redox cycling systems consisted of multiple enzymes and nanomaterials, coupled with series enzymatic, electrochemical and chemical reactions. Compared with enzymatic biosensors, whole-cell biosensors exhibit distinctive advantages as high stability, simple preparation procedures, and also reduced cost [29]. Thus, design and construction of whole-cell based redox cycling system is valuable for high sensitive riboflavin detection.

Theoretically, the whole-cell based redox cycling system can be realized in two manners based on whether the redox species are recycled via cell oxidation or reduction. Dissimilatory metal-reducing bacteria *Shewanella oneidensis* is the promising candidate for such redox cycling as it is capable of bidirectional extracellular electron transfer [30,31]. *Shewanella* cells could transfer the endogenous electron to the electrode via sequenced cytochromes including CymA, MtrA, MtrB and MtrC/OmcA and flavins when the positive electrode potential is poised (more positive than −0.4 V *vs.* SCE), known as outwards EET [32,33]. Meanwhile, this process could also be reversed and finally reduce the fumarate at cell periplasm with cathodic electron when negative electrode potential as −0.6 V (*vs.* SCE) was poised, which is known as inwards EET [30]. Remarkably, the bidirectional electron transfer between *Shewanella* cells and electrode is controlled by the redox potential shift of flavin when it interacts with outer-membrane cytochromes (MtrC/OmcA) [34]. Thus it is feasible to design *Shewanella* based riboflavin redox cycling system. In our previous work, we developed a riboflavin cycling system based on *Shewanella* inwards electron transfer, in which negative potential (−0.6 V *vs.* SCE) was selected to allow riboflavin to be reduced by the electrode. The reduced riboflavin then transferred the electron to *Shewanella* cells via its inwards electron transfer and finally reduced fumarate to succinate with the aid of fumarate reductase at *Shewanella* periplasm. This electrode reduction and cell oxidation process enabled riboflavin cycling, which increased the response current and allowed the development of a riboflavin amperometric sensor [23]. Here, we postulate that the *Shewanella* outwards electron transfer capability also can be adopted for riboflavin cycling system (by poising the electrode at positive electrode potential), in which the endogenous electron from *Shewanella* metabolism could be used to continuously reduce the electrode-oxidized riboflavin.

In this work, electroactive bacteria *S. oneidensis* MR-1 (served as the biocatalyst) and its outwards electron transfer capability was adopted to construct a whole-cell based riboflavin redox cycling system. CV analyses were conducted to study the effect of *Shewanella* cells and microbial endogenous electron on the electrochemical behavior of riboflavin. Meanwhile, the feasibility of using this redox cycling system integrated electrochemical cell for the stable amperometric discharge was investigated. Riboflavin amperometric sensor based on *Shewanella* outwards electron transfer was then developed. The detection range, sensitivity, selectivity and stability of this riboflavin amperometric sensor were studied. Furthermore, riboflavin in spiked authentic samples, pharmaceutical and food samples were tested using the developed sensing system to evaluate its potential application.

2. Materials and methods

2.1. Bacteria strains and cultivation conditions

Shewanella oneidensis MR-1 (ATCC 700550) was cultivated in LB broth (10 g/L tryptone, 5 g/L yeast extract (Oxoid, UK), 5 g/L NaCl, pH = 7.2) at 30 °C in a shaking incubator (ZWY-100H, Shanghai Zhicheng Analytical Instrument Manufacturing Co., Ltd., China) with 150 rpm [35,36]. The bacteria were harvested by centrifugation with 5000 rpm for 10 min (Avanti J-E, Beckman Coulter, USA) and washed with M9 salt medium (1 g/L NH_4Cl, 0.5 g/L NaCl, 17.8 g/L $Na_2HPO_4{\cdot}12H_2O$, 3 g/L KH_2PO_4, 0.0111 g/L $CaCl_2$, 0.12 g/L $MgSO_4$, pH = 7.0 [37]) for three times to remove bacterial metabolites and re-suspended in the fresh electrolyte (M9 salt medium with 10 mM sodium lactate as carbon source, bubbling nitrogen for 15 min before use) to desired density [38,39]. All chemicals were of analytical grade and were purchased from Sinopharm Chemical Reagent Co., Ltd. (China) unless otherwise indicated.

2.2. Electrochemical setup

All the electrochemical analyses were conducted with CHI660E electrochemical workstation (Chenhua Instruments Co., Ltd., Shanghai, China). Three-electrode electrochemical system assembled in a 15 mL cylindrical borosilicate glass bottle was adopted for electrochemical analyses and electrochemical biosensor measurement. Carbon cloth with geometry size of 1 cm × 2 cm was used as working electrode, a platinum wire electrode and saturated calomel electrode (SCE, +0.243 V *vs.* SHE) (Chenhua Instruments Co., Ltd., Shanghai, China) were used as counter and reference electrode, respectively. Bacteria suspended in fresh electrolyte (12 mL) were inoculated into the electrochemical cell for electrochemical characterization or biosensing. Cyclic voltammetry (CV) analyses were conducted from −0.8 V to 0.1 V with scanning rate of 5 mV/s. The amperometric analyses were conducted at the constant potential of 0 V with sample interval of 1 s. Constant slow rate magnetic stirring (200 rpm) was adopted during the electrochemical analyses. All the potential described in this work is versus