Glucose detection via Ru-mediated catalytic reaction of glucose dehydrogenase

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Abstract

In the electrochemical glucose sensor field, glucose dehydrogenase (GDH) has attracted attention as an enzyme alternative to glucose oxidase (GOD), which suffers performance issues due to variability in oxygen concentrations. The typical mediator used with GOD in electrochemical glucose sensors, hexamine ruthenium ([Ru $(NH_3)_6$]), has not been applied with GDH. Herein, a new mediator, [ruthenium (4,4′-dimethoxy-2,2′-bipyridine)$_2Cl_2$] ([Ru(dmo-bpy)$_2Cl_2$]), was synthesized and applied to facilitate electron transfer between GDH and the electrode. The prepared [Ru(dmo-bpy)$_2Cl_2$] was examined physicochemically by NMR, UV-vis, and XPS spectroscopy, and electrochemically by CV. Then, GDH and a cross linker, poly (ethylene glycol) diglycidyl ether, were adsorbed with [Ru(dmo-bpy)$_2Cl_2$] onto a screen-printed carbon electrode. The glucose response of [Ru(dmo-bpy)$_2Cl_2$] with GDH as an electron-transfer mediator was investigated by potentiostat. The resulting electrical currents were well correlated (R^2 = 0.9984) with glucose concentration (5.0, 10.0, 15.0, and 30.0 mM). Therefore, this ruthenium complex can be used for glucose detection with GDH as a good substitute mediator. Copyright © 2018 VBRI Press.

Keywords: Mediator, glucose dehydrogenase, glucose sensor, coordination complex.

Introduction

Electrochemical glucose sensors, first proposed by Clark and Lyons in 1962 [**1**], can be classified into three generations [**2**]. First-generation sensors measure the hydrogen peroxide produced by the action of GOD, glucose, and dissolved oxygen. However, human *in vivo* oxygen concentrations can differ depending on the individual, their environment, or gender. Accordingly, second-generation electrochemical glucose sensors that use a mediator to boost the efficiency of electron transfer were developed. Mediated electron transfer is more efficient and faster than the first-generation process because the mediator transfers electrons from the enzyme, which is reduced by glucose, to the electrode. The key improvement in third-generation glucose sensors is the transfer of the electron directly to the electrode without a mediator. This type of system has higher technical requirements because it has lower reproducibility, and is difficult to manufacture. For these reasons, most producers fabricate second-generation glucose sensors that employ a mediator [**3**−**5**].

Mediators based on Fe, Ru, and Os species have been used in second-generation glucose sensors. Ruthenium hexamine ([Ru$(NH_3)_6$]) is employed as a mediator for the enzyme glucose oxidase (GOD) by most manufacturers [**6**−**8**]. However, it has become increasingly apparent that the oxygen-sensitivity of the GOD enzyme in second-generation glucose sensors is a problem. As a consequence, an alternative, oxygen-insensitive enzyme, glucose dehydrogenase (GDH), is being considered for use in the determination of glucose as a second-generation glucose sensor [**9**−**11**].

Recently, GDH-responsive mediators such as organic compound, Os, and Ru compounds have been studied to facilitate electron transfer between the enzyme and electrode [**12**−**15**]. In this study, hexacoordinate [Ru (dmo-bpy)$_2Cl_2$] was synthesized via the coordination of 4,4′-dimethoxy-2,2′-bipyridine with [$RuCl_3$]. The physicochemical and electrochemical characteristics of [Ru (dmo-bpy)$_2Cl_2$] were investigated by NMR, UV-vis, XPS, and CV. Then, the catalytic reaction of [Ru (dmo-bpy)$_2Cl_2$] with GDH at various glucose concentrations was evaluated by CV. The synthesized [Ru(dmo-bpy)$_2Cl_2$] demonstrates good potential for use as a mediator with GDH in second-generation electrochemical glucose sensors.

Experimental

Reagents and materials

Ruthenium(III) chloride hydrate, 4,4′-dimethoxy-2,2′-bipyridine (dmo-bpy), D-(+)-glucose, poly(ethylene glycol) diglycidyl ether (PEGDGE), and other chemicals were purchased from Sigma-Aldrich Co. (Milwaukee, WI, USA). Glucose dehydrogenase (FAD-dependent GDH-8, 206 U/mg) was purchased from Amano Enzyme Inc. (Japan). Phosphate buffered saline solution (PBS: 4.3 mM NaH_2PO_4, 15.1 mM Na_2HPO_4, 140 mM NaCl) and all other solutions were prepared using deionized Milli-Q water (Milli-Q® Academic, Molsheim, France). All chemicals were analytical grade.

Synthesis of [Ru(dmo-bpy)$_2$Cl$_2$]

$[Ru(dmo\text{-}bpy)_2Cl_2]$ was synthesized by adapting a previously reported method [16]. $RuCl_3{\cdot}xH_2O$ (35.1 mg, 0.1692 mmol) and dmo-bpy (73.18 mg, 0.3384 mmol) were heated to reflux in anhydrous ethanol (40 mL) for 1.5 h **(Fig. 1).** After cooling to room temperature, the crude product was isolated by the dropwise addition of the reaction mixture into diethyl ether (500 mL) followed by filtration of the precipitate through a 0.45 μm membrane filter. Then, the product was purified by column chromatography over aluminum oxide with ethanol, followed by re-precipitation through dropwise addition of the concentrated solution into diethyl ether (500 mL). Finally, after filtration (0.45 μm membrane filter), the brown powder was dried in a vacuum oxen at 60℃ for 1 day.

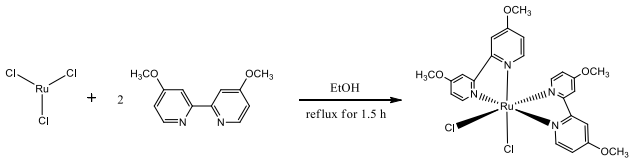

Fig. 1. Synthetic scheme for $[Ru(dmo\text{-}bpy)_2Cl_2]$ mediator.

Characterization of [Ru(dmo-bpy)$_2$Cl$_2$]

The successful synthesis of $[Ru(dmo\text{-}bpy)_2Cl_2]$ was confirmed by ^{1}H NMR spectroscopy (Varian Ascend 500, 500 MHz). The conjugation of the synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ was verified by UV-vis spectroscopy (Model 8453, Agilent Technologies, Shanghai, China) and X-ray photoelectron spectroscopy (XPS, SPECS, Berlin, Germany) at 3.5 kV and a scan number of 7.

Electrochemical measurements

All electrochemical experiments were carried out with CHI 660B software (CH Instruments, Inc., Austin, TX, USA). A prepared screen-printed carbon electrode (SPCE, diameter = 3.5 mm) was used as the working electrode. A micro Ag/AgCl (3.0 M KCl, Cypress, Lawrence, KS, USA) scrolled with a 0.5 mm diameter platinum wire was used as the reference and counter electrodes, respectively.

To prepare the working electrodes, the GDH (200 mg/mL in PBS, 4 μL) was adsorbed with $[Ru(dmo\text{-}bpy)_2Cl_2]$ (10 mg/mL in DW, 4 μL) and PEGDGE (10 mg/mL in DW, 1 μL) onto the SPCE by micropipette and dried in on oven at 36 °C for 12 h. Also, the synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ were fabricated under the same conditions as above. The electrical responses of the enzyme-adsorbed SPCE were measured by CV in 40 μL glucose dissolved in 1X PBS. CV experiments were performed to investigate the response to various glucose concentrations (1.0, 5.0, 10.0, 15.0, and 30.0 mM) at 0.3 V vs. Ag/AgCl.

Results and discussion

Physicochemical characterization of [Ru(dmo-bpy)$_2$Cl$_2$]

The synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ was fully characterized by ^{1}H NMR spectroscopy in pyridine-d_5. The signals of the complex displayed measurable Ru coupling by virtue of their proximity to the metal-coordinated nitrogen [17]. As shown in **Fig. 2(a),** the proton signals for the free dmo-bpy are observed at δ 8.6395 (d, 1H, J = 5.5, pyridine), 8.4255 (s, 1H, pyridine), 6.9400 (d, 1H, J = 5.5 pyridine), and 3.7400 (s, 3H, $–OCH_3$) ppm. After complexation, the ligand proton resonances are shifted to δ 9.302 (s, 1H, pyridine), 7.990 (s, 1H, pyridine), 7.113 (s, 1H, pyridine), and 4.273 (s, 3H, $–OCH_3$) ppm **(Fig. 2(b))**, confirming ligation of the dmo-bpy ligand to the Ru center.

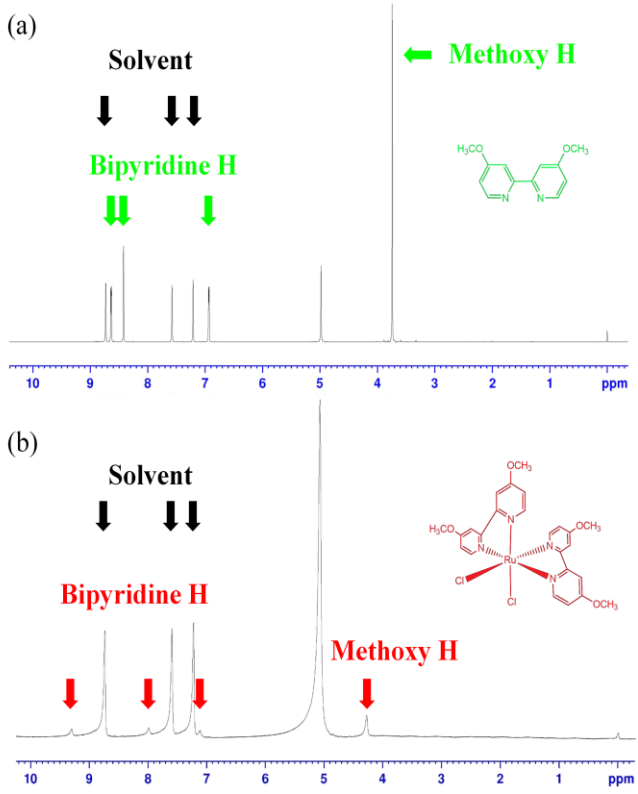

Fig. 2. NMR spectra of the (a) dmo-bpy ligand and (b) $[Ru(dmo\text{-}bpy)_2Cl_2]$ mediator.

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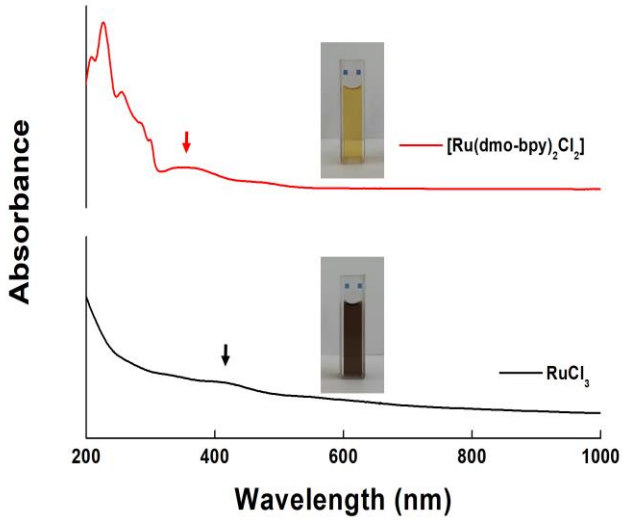


Fig. 3. UV-vis spectra of $RuCl_3$ (black line) and $[Ru(dmo\text{-}bpy)_2Cl_2]$ (red line).

The direct conjugation of dmo-bpy was studied via UV-vis spectroscopy, as shown in **Fig. 3,** in which the UV-vis spectra of $RuCl_3$ (black line) and $[Ru(dmo\text{-}bpy)_2Cl_2]$ (red line) dissolved in water (1 mM) are displayed. The UV-vis spectra of $RuCl_3$ and $[Ru(dmo\text{-}bpy)_2Cl_2]$ exhibit an absorption peak at 349 nm, while the characteristic *d-d* absorption band at 390 nm for $RuCl_3$ disappears [18]. Overall, the UV-vis spectrum of $[Ru(dmo\text{-}bpy)_2Cl_2]$ is similar to that previously reported for $[Ru(dmo\text{-}bpy)_3]$ [19]. Also, the peaks in the ultraviolet range from 208 to 298 nm are typical for the pyridine peaks of dmo-bpy.

To verify the coordination of $RuCl_3$ and 4,4′-dimethoxy-2,2′-bipyridine, XPS was performed. The typical detailed scan from the XPS analysis of dmo-bpy (green line) shows the nitrogen 1*s* peak of pyridine at 401.4 eV in **Fig. 4(a)**. Upon coordination, the nitrogen 1*s* peak in $[Ru(dmo\text{-}bpy)_2Cl_2]$ (red line) is shifted to a binding energy of 400.0 eV. Also, the typical Ru binding energy plot for $RuCl_3$ **(Fig. 4(b))** reveals two distinct peaks for the Ru($3d_{3/2}$) and Ru($3d_{5/2}$) orbitals at 285.15 and 282.5 eV, respectively. However, the Ru($3d_{3/2}$) binding energy in the synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ shifts to 285.7 eV. Also, the Ru($3d_{5/2}$) peak for $[Ru(dmo\text{-}bpy)_2Cl_2]$ is decreased in intensity compared to the peak for $RuCl_3$ [18,20,21]. From these results, the synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ may be characterized as a well-coordinated redox complex.

In the CV of $[Ru(dmo\text{-}bpy)_2Cl_2]$ (1.0 mg/mL in 0.1 M PBS (pH 7.4)) on the SPCE, we observe well-defined redox peaks ($E_{1/2}$ = 0.206 V vs. Ag/AgCl), as shown in **Fig. 5**. The coordinated $[Ru(dmo\text{-}bpy)_2Cl_2]$ (red line) shows more stable oxidation and reduction peaks than the $RuCl_3$ precursor (black line). This suggests that $[Ru(dmo\text{-}bpy)_2Cl_2]$ can function as a fast and reversible redox mediator that could be used for electrochemical glucose detection as second-generation mediator.

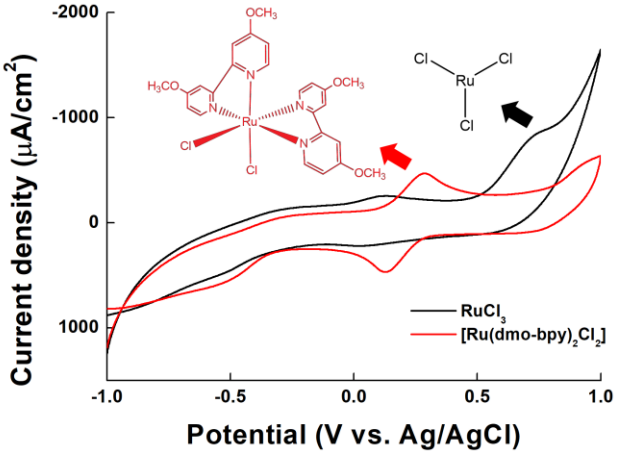


Fig. 5. CVs of the precursor $RuCl_3$ (black line) and synthesized $[Ru(dmo\text{-}bpy)_2Cl_2]$ (red line).

Glucose measurements

The electron-transfer interactions between GDH and the mediators $[Ru(NH_3)_6]$ and $[Ru(dmo\text{-}bpy)_2Cl_2]$ were investigated by CV. As shown in **Fig. 6(a),** the GDH electrode with the $[Ru(NH_3)_6]$ mediator does not react appreciably with 30 mM glucose (red line) compared to 0 mM glucose (black line). However, the GDH electrode

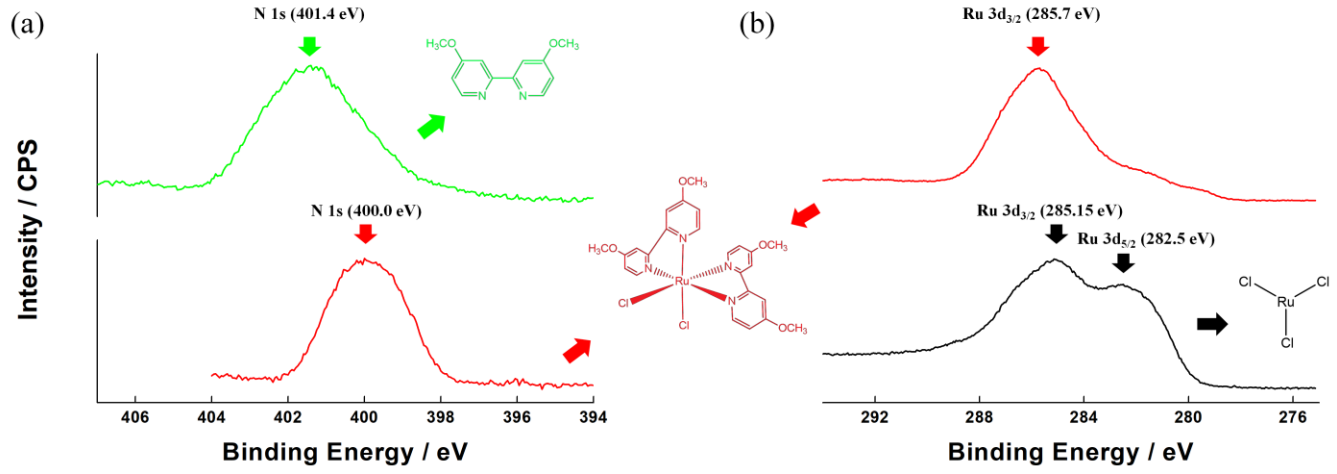
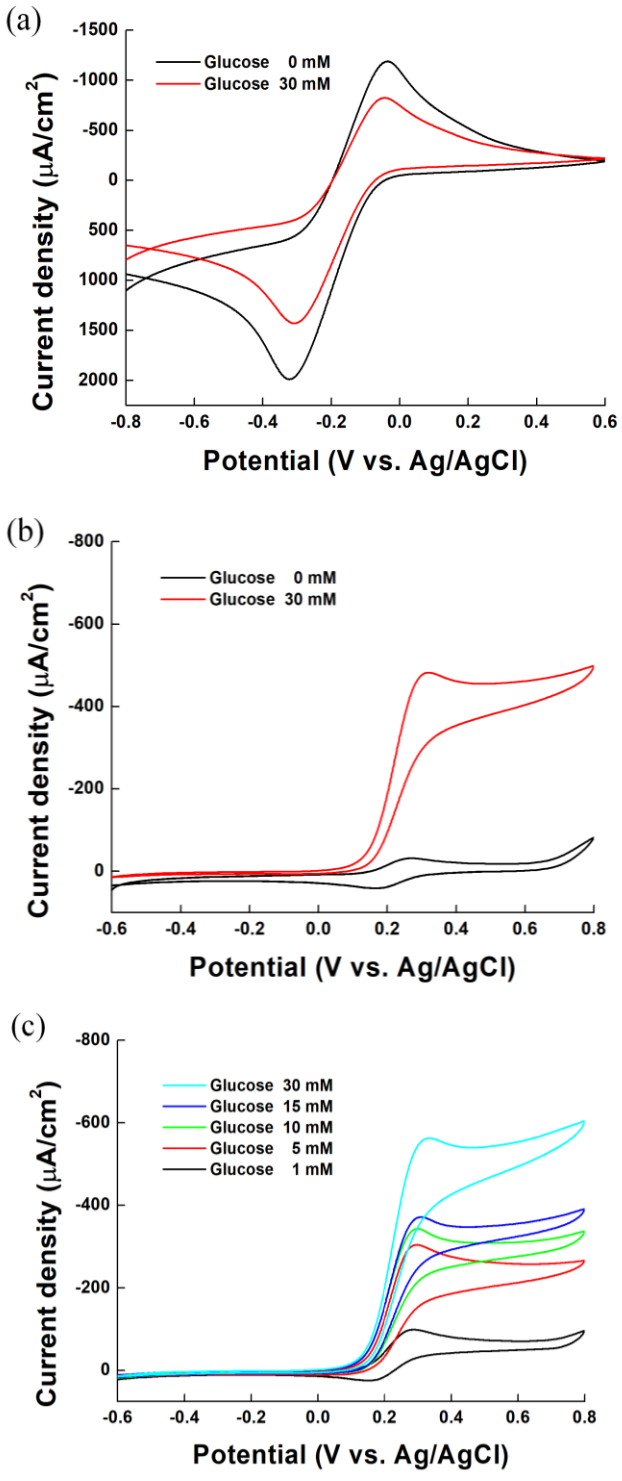


Fig. 4. XPS spectra in the binding energy ranges of N 1s and Ru $3d_{3/2}$ and $3d_{5/2}$

modified with the synthesized [Ru(dmo-bpy)$_2$Cl$_2$] exhibits a dramatically increased current at 30 mM glucose (red line) compared to 0 mM glucose (black line) (**Fig. 6(b)**). The results suggest that the synthesized [Ru(dmo-bpy)$_2$Cl$_2$] can be used as a GDH mediator. CV was next used to determine glucose levels. **Fig. 6(c)** shows the CVs of the catalytic currents on the enzyme-adsorbed SPCE in the presence of various concentrations of glucose (1.0, 5.0, 10.0, 15.0, and 30.0 mM). The anodic peaks increase linearly with successive increases in the glucose concentration (5.0, 10.0, 15.0, and 30 mM).



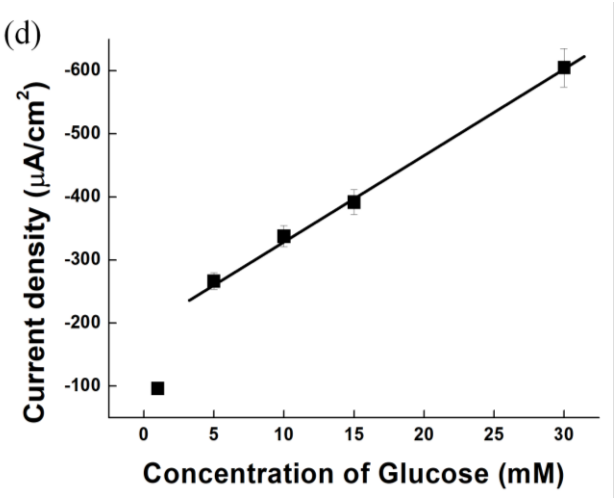


Fig. 6. CVs of (a) [Ru(NH$_3$)$_6$] and (b) [Ru(dmo-bpy)$_2$Cl$_2$] at 0 mM (black line) and 30 mM (red line) glucose. (c) CVs and (d) concentration curve of the synthesized [Ru(dmo-bpy)$_2$Cl$_2$] with GDH at different glucose concentrations (1.0, 5.0, 10.0, 15.0, and 30.0 mM).

Fig. 6(d) shows the linear dependence of the anodic peak current with the glucose concentration in the range of 5.0–30.0 mM, with a correlation coefficient of 0.9984(R^2). Also, the limit of detection (LOD) is determined as 1.21 mM, with an RSD of 4.25% (N = 10, where N denotes the number of different electrodes used). The catalytic current obtained from our new synthesized mediator is 605.67 mA/cm^2 at 30.0 mM glucose.

Conclusion

Mediators that can respond with oxygen-insensitive GDH in second-generation glucose biosensors are urgently needed. The widely used hexamine ruthenium does not catalyze the reactions of GDH even at high current levels. In this work, we synthesized the [Ru(dmo-bpy)$_2$Cl$_2$] complex, which does react with GDH. The coordinated [Ru(dmo-bpy)$_2$Cl$_2$] complex was characterized by NMR, XPS, UV-vis, and CV. Additionally, the [Ru(dmo-bpy)$_2$Cl$_2$] complex was demonstrated to be a fast and reversible redox mediator by CV. Finally, through CV analysis, the linearity of the oxidation currents over a wide glucose concentration range reveal the complex as a good mediator. Our synthesized ruthenium-based mediator has the potential to become a good substitute for currently used industrial biosensor mediators.

Acknowledgements

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